

Objective

This application guide describes how to capture video using the BehaviorCloud Camera app and generate tracked video traces and tabulated data including ambulatory vs. resting time, distance traveled, velocity, time spent in zones, zone latencies, number/sequence of zone entries, and spontaneous alternation.

Common use cases include: testing rodent, zebrafish, or insect behavior in mazes and other enclosures (e.g., elevated plus maze, open field, Y-maze, T-maze, novel object recognition, Barnes maze, etc.)

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1. Creating an Account

To get started you will need a BehaviorCloud account. Set up an account at the <u>BehaviorCloud</u> website using your institutional/company email address. You will receive an email prompting you to validate your email address and sign in to your new account.

2. Capturing Video

a. Downloading the Camera app

Download the free BehaviorCloud Camera app by visiting <u>iTunes</u> on your mobile device. Compatible iOS devices include: iPhone, iPad, or iPod Touch. You can purchase a video recording bundle, including a current-generation iPod Touch and mounting hardware, from our online store.

b. Streaming Video

Capturing video with the BehaviorCloud Camera app requires a stable WiFi connection for streaming and a power source for your mobile device. Log in to the BehaviorCloud Camera app using the credentials you created above and mount the mobile device above your cage or maze so the desired recording area is in view. The mobile device running the Camera app serves as the video camera - you will need a second mobile device or laptop computer to control your experiment.

Make sure the phone is mounted securely so it will not shift during testing. Smartphone mounts or tripods work well for this purpose.

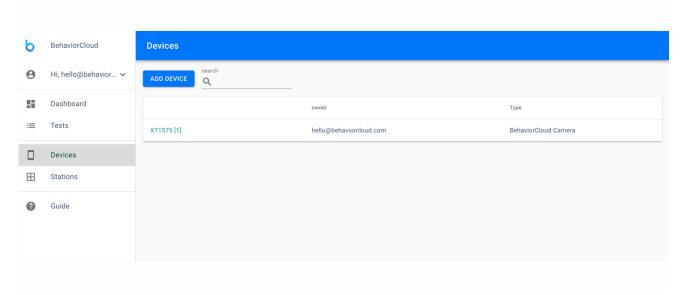
The ambient lighting in the testing room must be sufficient for the camera to capture clear video. If testing in dim light or darkness is required, then a red light or, depending on particular device specifications, an infrared light source can be used to capture clear video.

Set up the experiment in a low traffic area. Shadows moving across the tracking area may interfere with tracking of the subject or affect behavior of the test subjects.

Streaming works best if the Camera app is positioned to record in landscape orientation - not portrait.

The first time you log in to your account you will see options to add a device or create a test. Make sure you have installed the BehaviorCloud Camera app onto the mobile device you intend to use for recording video (as described above). The first time you log in to the mobile app it will be automatically paired to your account. Check in the Devices page to make sure your mobile device is listed.



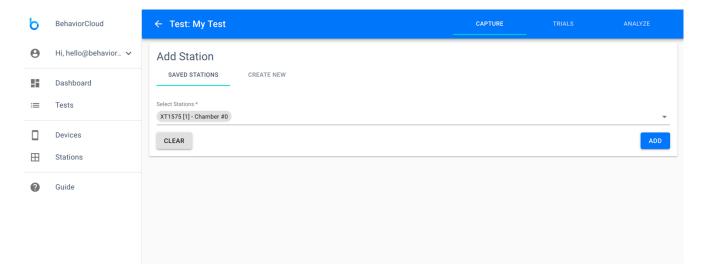


Navigate to Tests and click Create Test. Enter a name and select the type of test from the dropdown menu. For video data, you should choose either Automated Video Tracking or Manual Video Scoring, depending on your primary analysis strategy. You will have the option to add both analyses later if desired (described more below). After selecting the type of test, you will be prompted to enter the duration of the test in seconds.

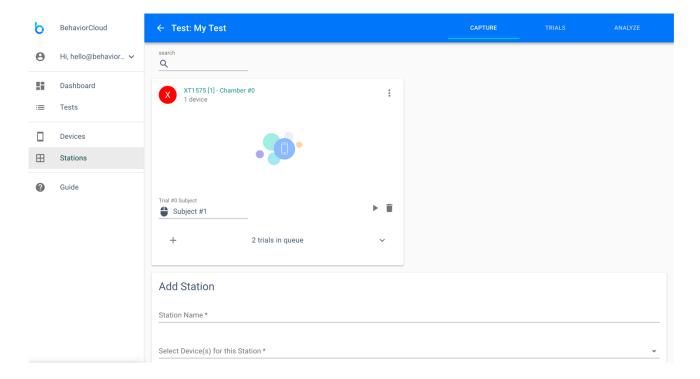


The next screen asks if you would like to capture data or upload data. Click capture data. This takes you to a page for adding stations to your test. A station (sometimes known as a test chamber) is a data-capture set-up consisting of one or multiple data sources. In this case, the station is the mobile device you have connected for capturing video, likely mounted above a maze or cage. If you had multiple mobile devices that you planned to run at the same time (e.g., for running multiple subjects in separate mazes), then you could add them all on this page as individual stations.



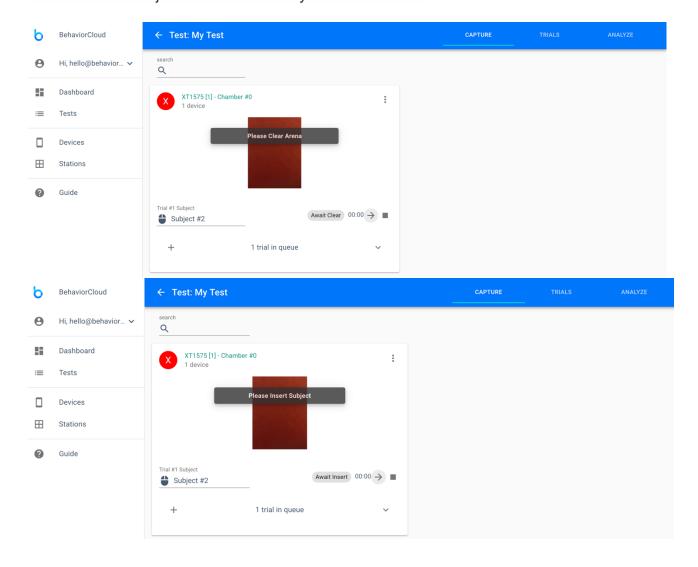


After adding your station(s) you will see your main working view for capturing data. Note the three tabs at the upper right of the screen. These tabs allow you to toggle between Capture, Trials, and Analysis views for a given test. In the Capture view, each station is represented by a card. Clicking the plus icon at the bottom left of a station card will allow you to add subjects to the queue for that station. Clicking the play icon to the right of a subject will start a trial.

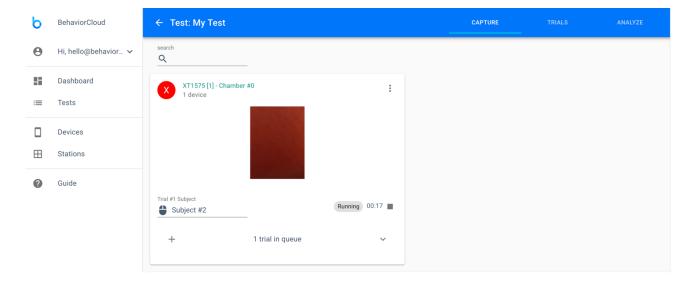




After starting a trial you will see a live preview of the video stream. A prompt will ask you to clear the arena, meaning remove all subjects, shadows, movements etc. from the camera view to provide a clean background image. Click the arrow to proceed when ready. Another prompt will ask you to insert the subject. Click the arrow when ready to begin the trial. When time is up, the trial will automatically end and you will be prompted to enter the next subject. Record as many trials as desired.

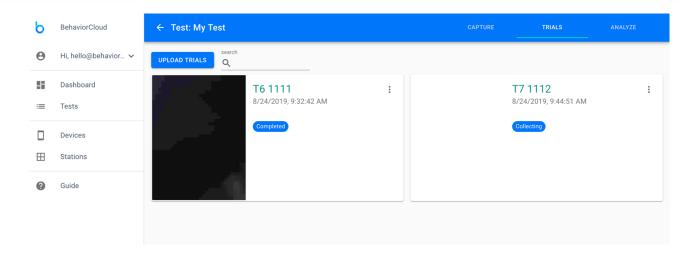






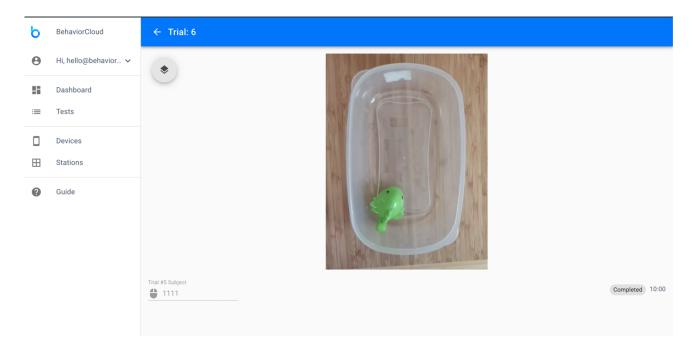
You can also click on a station to go to a more detailed view. If you're running one station at a time, then the detailed view is useful. If you're running multiple stations at the same time, the main Capture view shown in the images above is useful for previewing and controlling all your stations at once.

Navigate to the trials page to see a list of your completed and currently running trials.



Click on a completed trial to see the detail view for that trial. On this page, you can play back your trial or add additional meta-data. Click the overlay icon in the upper left to see options to add a distance calibration, zone map, tracking boundary, or manual scoring data. We'll cover these options in more detail below.

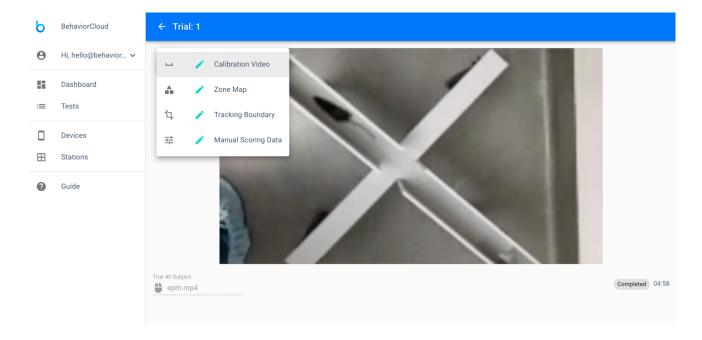




3. Configuring Trials

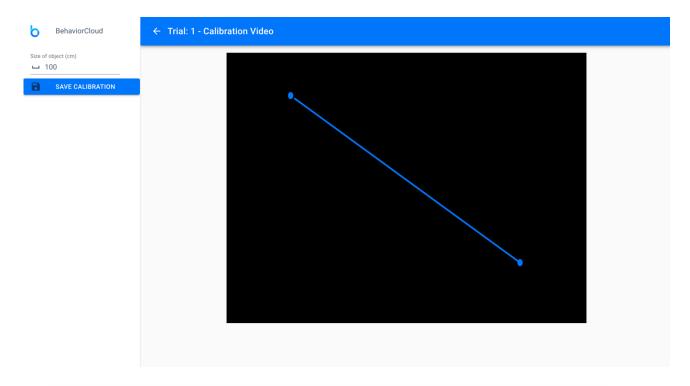
a. Distance Calibration

In the detail view for one of your trials, click the overlay icon and then click on the pencil icon next to Calibration.





Drag the line to represent a length of known distance and enter that measure in centimeters in the left-hand menu. Click Save. You will be asked if you would like to apply this calibration to any other trials. If you have captured multiple trials using the same maze/cage you can use this function to quickly select all of those trials and apply your calibration across all of them.

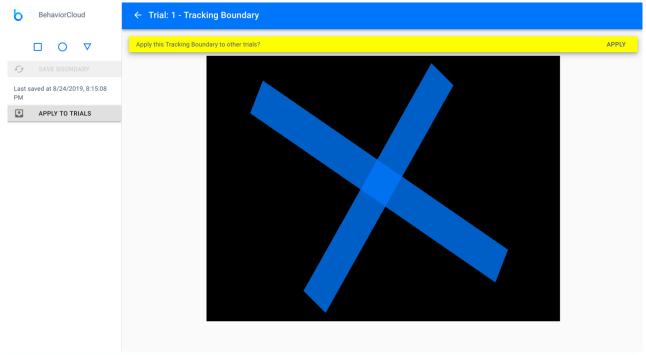


The calibration that you set in this step will be used for downstream analysis to calculate distance traveled, velocity, etc.

b. Tracking Boundary

Back on the trial detail view, click the pencil icon next to Tracking Boundary. The tracking boundary is important for automated video tracking analysis because it tells the system what areas of the video frame to use for tracking analysis vs. what areas to exclude. This is especially important if there are other subjects or people moving around in the areas adjacent to the maze/cage you are tracking.





Use the provided shape tools to cover the area you would like to track. Anything outside of this will be excluded from the automated video tracking analysis. Click save and then apply to other trials if desired.

c. Zone Map

Similar to the tracking boundary, the zone map is used for automated video tracking analysis to distinguish certain areas of a maze or cage for independent analysis. In the zone map view, use the provided shape tools to cover the areas you would like to set as zones. Name your zones if desired. These names will be carried through to the analyzed results output by BehaviorCloud. Click save and then apply to other trials if desired.





4. Analyzing Data

Once you have captured all of your trials and configured them as needed, you can check that everything is complete and correct by visiting the Trials tab of your test. Each trial is shown as a separate card with a thumbnail video preview that includes any tracking boundaries and/or zone maps that you have set, and a list of all of the associated meta-data with that trial (e.g., distance calibration). You can also see if any initial analyses are already underway. BehaviorCloud begins to generate raw position data for automated video tracking experiments as soon as the calibration is set.

a. Tracked Position Video

The tracked position video is a duplicate of your captured video with a mask applied to show the centroid position being tracked for analysis. This video will appear in the overlay menu on each trial's detail view when it is ready. It is automatically generated as soon as a calibration and tracking boundary is available. The tracked position video for each trial is also available in the Analysis tab.

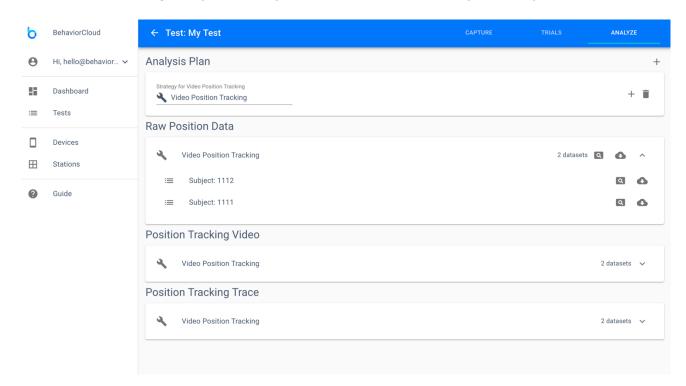
b. Tracked Position Trace

The tracked position trace is an image showing the entire path that was tracked during the course of the trial. This image will appear as an option to view in the overlay menu on each trial's detail view when it is ready. It is automatically generated as soon as a calibration and tracking boundary is available. The tracked position trace for each trial is also available in the Analysis tab.



c. Analysis Plan

Navigate to the Analysis tab and you will see a space for your Analysis Plan at the top of the page. This is where you can choose the relevant analyses for your test. Use the plus icon to add an analysis. It will begin processing as soon as it is added. Use the trash icon to cancel or delete an analysis. The results of your analyses will appear in the drawers below your plan. You can expand those drawers to see which results are ready, which results are still being analyzed, and you can view or download your analyzed data.



d. Activity Analysis

Select Activity Analysis to generate activity-related variables for each subject. BehaviorCloud will use the raw position data generated for each video from the Position Tracking Video to calculate distance traveled, ambulatory time, resting time, and average velocity.

e. Zoned Activity Analysis

Select Zoned Activity Analysis to generate zoned activity-related variables for each subject. BehaviorCloud will use the raw position data generated for each video from the Position Tracking Video along with the zone map you configured above to calculate distance traveled, ambulatory time, resting time, and average velocity for each zone. It will also output the latency to enter each zone.



f. Spontaneous Alternation

Spontaneous Alternation is an analysis mainly used for Y-maze and T-maze videos. This will calculate the percent alternation for each trial (number of triads divided by number of arm entries minus two).

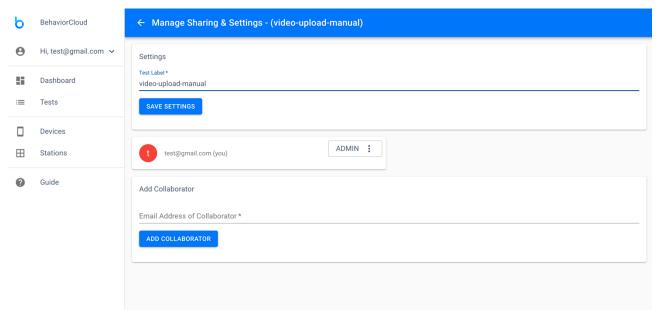
g. Zoned Sequence Analysis

Select Zoned Sequence Analysis to generate a list providing the sequence of zone entries for each trial.

5. Sharing a Test

a. Adding Collaborators

You can share your data and invite collaborators to contribute to any phase of the experiment through your BehaviorCloud account. Go to Tests to view all of your experiments. Private experiments are indicated by a lock icon and shared experiments are indicated by a share icon. To add a collaborator, select the three dots menu icon to the right of a test and click Manage Sharing and Settings. You will be able to enter the email address of the person you would like to invite to your test. Your collaborator will receive an email notification prompting them to login and access the shared experiment.



b. Managing Permissions

The default level of permission is read only. You can manage their level of permission: read only (can view data but cannot edit), read/write (can view, contribute, and edit), or admin (full permission, including to delete an experiment).



6. Downloading Data

All of your data are available for download at any time. Your data come in multiple forms depending on the analyses that were run. The full tracked video showing centroid position and the subject outline is available from the trial detail view by clicking the overlay icon and then clicking Position Tracking Video. The path tracked during the course of the trial can also be found in the overlay icon under Position Tracking Trace. The trace can be toggled on and off in overlay mode to be shown on the video background if desired. You can also access and download these data from the analysis tab of your test. Other data generated such as durations, counts, etc. are available after running them on the Analysis tab.

Additional Information

Further details can be found in our User Guide at www.behaviorcloud.com/guide.html

For support and inquiries, contact support@behaviorcloud.com