MOTION TRACKING SMALL ORGANISMS

A DMC GUIDE

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INTRODUCTION

This guide is intended for those interested in tracking the movement of small organisms, such as worms, hydra, etc. without prior experience with motion tracking and/or who do not have specialized software and equipment.

Both the software and equipment used in this guide are available from the DMC. **Adobe After Effects** is the software used to perform 2D motion tracking in this guide. A separate guide on Matlab's motion tracker may help those familiar with Matlab.

The results of Adobe After Effect's tracker will provide you with data on the frame-by-frame pixel coordinates of the tracked subject(s). The tracker works with single and multiple subjects. While this guide is focused on tracking small organisms (and hydra will be used as an example), the same process can be used to track other objects.

This guide is divided into three main sections: footage collection, preparing footage, and motion tracking.

CONTENTS

CLICK ON EACH HEADING TO
JUMP TO THAT SECTION

FOOTAGE COLLECTION TIPS

MOST IMPORTANT TIP 💡

Make sure the camera is **100% steady** during recording

- Most tracking software have issues when the footage is not taken from a **stationary camera.** Even tiny movements, such as a gradual shifting of the camera, will make tracking more difficult.
- Tighten all the screws on the tripod.
- Avoid unnecessary movement near the camera.

FOR SLOW MOVING ORGANISMS:

Use a Canon DSLR for incremental photos

- 0.2 fps can be taken using EOS Utility, connected to a PC
- 1 fps requires downloading Magic Lantern
- Manually focus

FOR FAST MOVING ORGANISMS:

Use a camcorder for video (30 fps)

- Manually focus

CHOOSING A BACKGROUND

Whenever possible, choose a **solid** colored background that **contrasts** with your subject. For example, if you are recording dark colored worms, choose a solid white background.

KEEP LIGHTING CONSTANT

- Make sure your subjects are well-lit.
  - Dim lighting = high ISO = grainy images
- Make sure the lighting does not change over time.

SAMPLE SETUP

Footage of hydra (10 mm organisms) were collected using the following setup:

- Canon EOS Rebel T3i DSLR with EFS 18-55mm f/3.5-5.6 IS lens

    Resolution: 5184 × 3456
    f/7.1
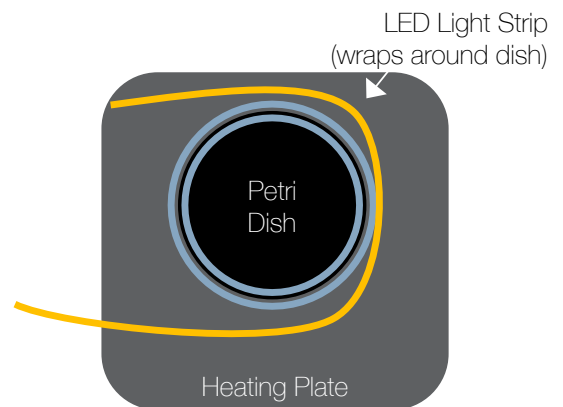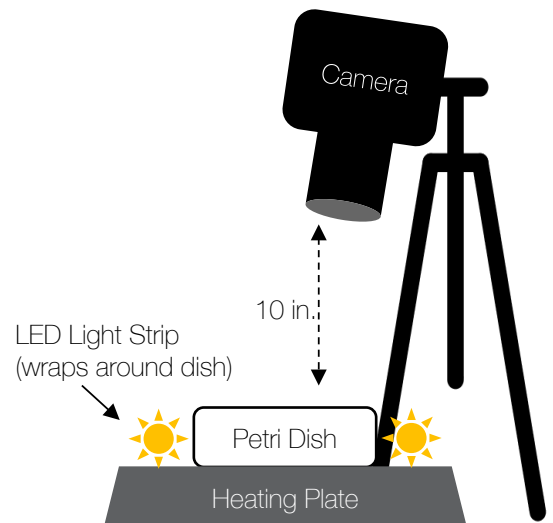   Exposure1/160
    ISO 1600

- Flexible LED light strip

- Standard tripod

The hydra are contained in the petri dish. The camera's distance from the petri dish is the minimum focusing distance for the particular lens used.
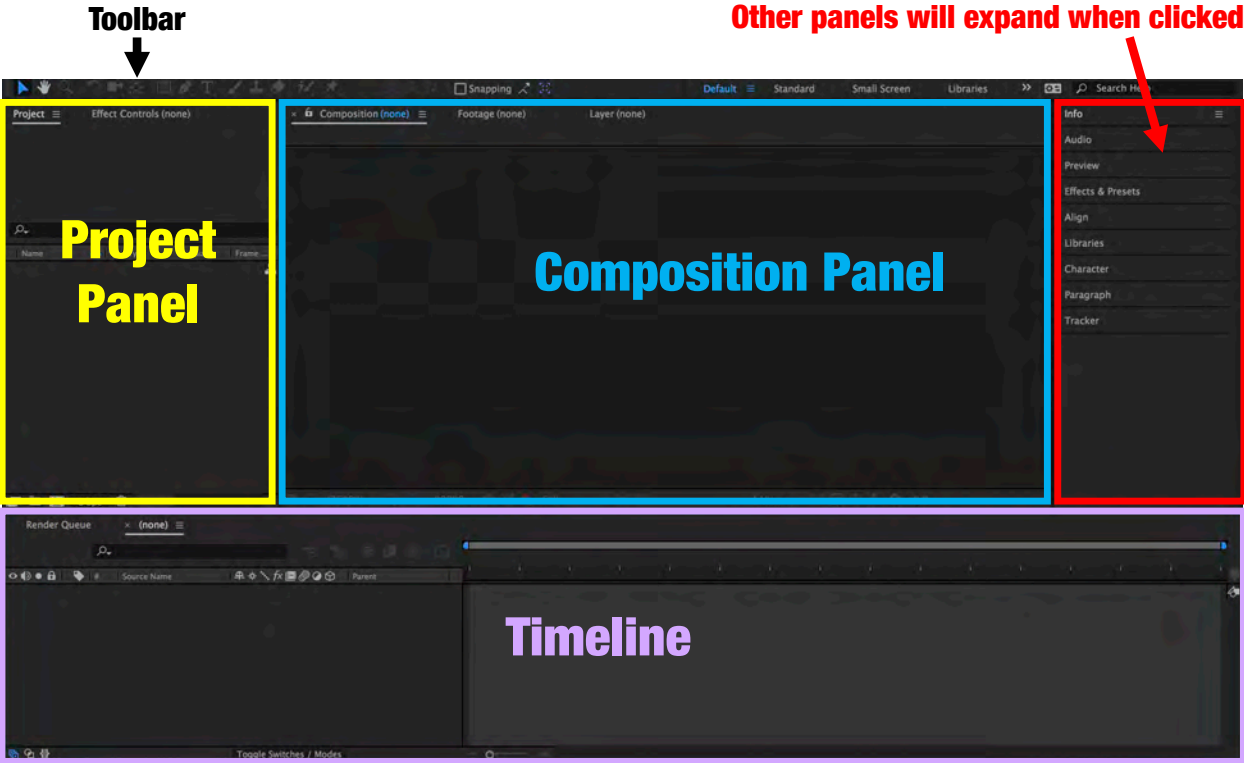
Top View

LED Light Strip
(wraps around dish)

Petri Dish

Heating Plate

Side View

Camera

10 in.

LED Light Strip
(wraps around dish)

Petri Dish

Heating Plate

PREPARING FOOTAGE IN
ADOBE AFTER EFFECTS

USER INTERFACE

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Toolbar

Other panels will expand when clicked

Project Panel

Composition Panel

Timeline

Toolbar Shortcuts:

- Selection Tool (V)
- Hand Tool (H)
- Zoom Tool (Z)
- Rotation Tool (W)
- Pen Tool (G)
- Type Tool (Cmd+T)

IMPORTING

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Organize Your Files
Create a folder for your footage. Make sure you create this folder in a location that will be stored if you are using a shared computer.

New Project
1. Open After Effects (AE)
2. Create a New Project
3. Click "File" > "Save As…" at the top of the window
   - Name your project
   - Choose the folder you created earlier with your footage
   - Click the "Save" button

Import
1. Double-click in the Project Panel to open the file browser*
2. Select your video file OR an image sequence
   - To import an image sequence, click "Options" and make sure the "Importer JPEG Sequence" box or "PNG Sequence" box is checked
   - Only the first image needs to be selected
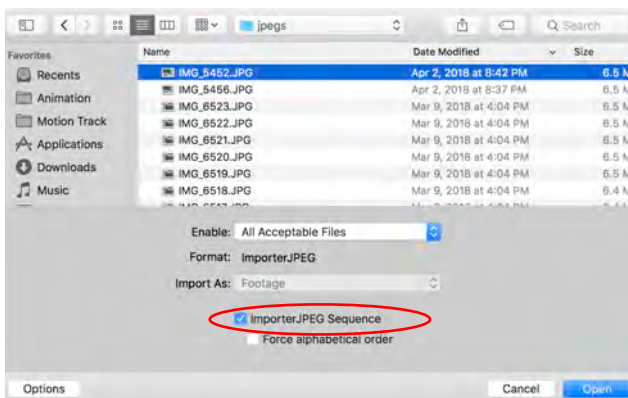
Note: "Imported" files are referenced by the AE project file. The files are NOT copied to your project location. If you change the location of the original files, AE cannot load the video properly.
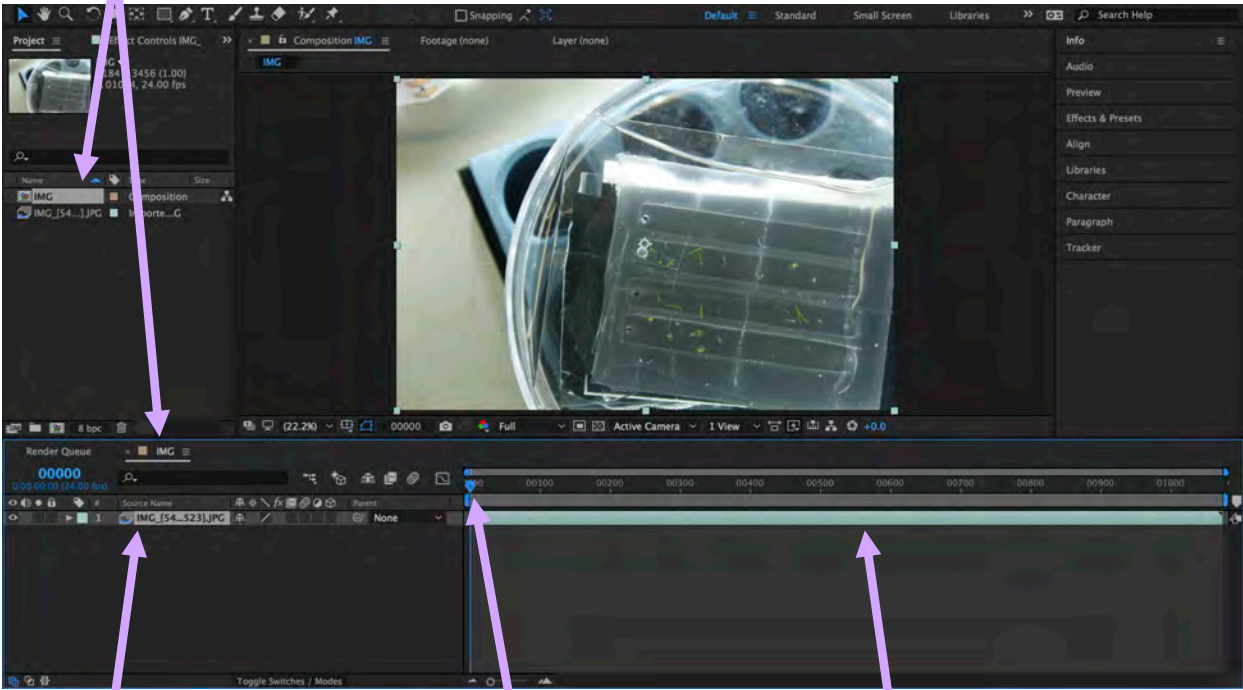
*Alternatively, you can drag-and-drop media directly into the Project Panel

CREATE A COMPOSITION

1. Select the footage you imported
2. Right-click
3. Choose "New Comp from Selection"
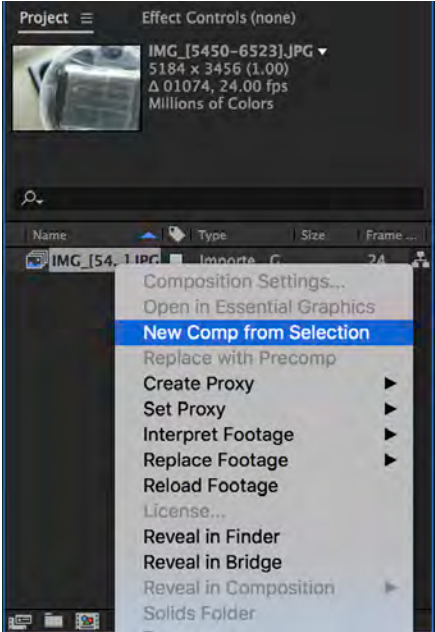
New composition

Contents of the Composition

Move the Playhead to scrub through the video

Contents of the Composition are loaded into the Timeline

SCALING & ROTATING

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To center footage and fit to frame

- Use the "Selection Tool" (V) to click on the footage in the Composition Panel. A selection box should appear around the footage.

- **Click** on the corner of the selection box and **drag** to scale the image. **Hold Shift** to scale proportionally.

To rotate footage

- Use the "Rotation Tool" (W)

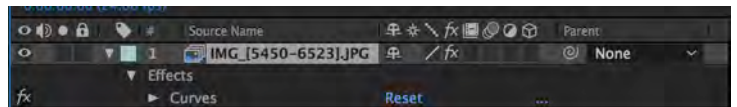- **Click and drag** in the Composition Panel to rotate the image

Alternatively, directly change the values under the "Transform" property of the layer in the Timeline
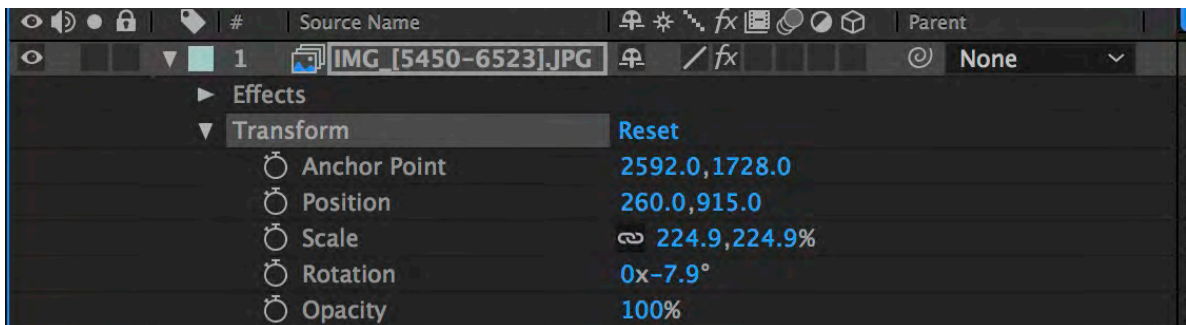
ADJUST CURVES

GOAL: Add more tonal contrast to your footage to make your subject(s) stand out from the background.

1. Make sure your footage is selected

2. Click on the Effects Panel > Color Correction > Curves

3. Click and drag the curves on the graph to adjust the tonal range. You can also change the channel you want to adjust.
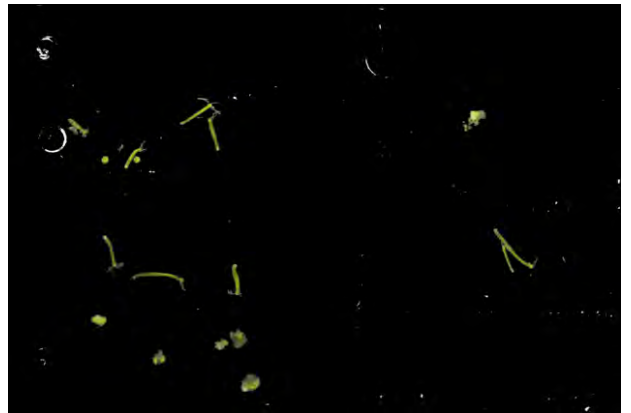
LINEAR COLOR KEY

Optional: Linear Color Key effect

If your footage does not have a solid background, the Linear Color Key effect can help improve the tracker's accuracy by subtracting out the background based on a selected background color.
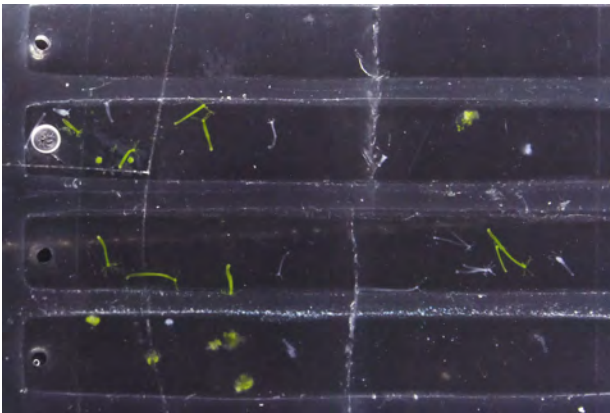
Without Linear Color Key

With Linear Color Key

Use the eyedropper tool to select a color from the background

Adjust these settings until background is sufficiently removed
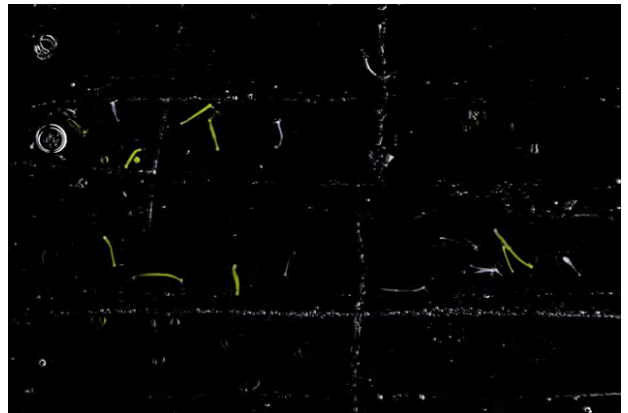
Optional: Difference Matte effect

If your footage does not have a solid background, a difference matte can help improve the accuracy of the tracker.

What is the Difference Matte effect?

*"The Difference Matte effect **creates transparency** by comparing a source layer with a difference layer, and then keying out pixels in the source layer that match both the position and color in the difference layer. Typically, it's used to **key out a static background** behind a moving object, which is then placed on a different background."   - AE User Guide*
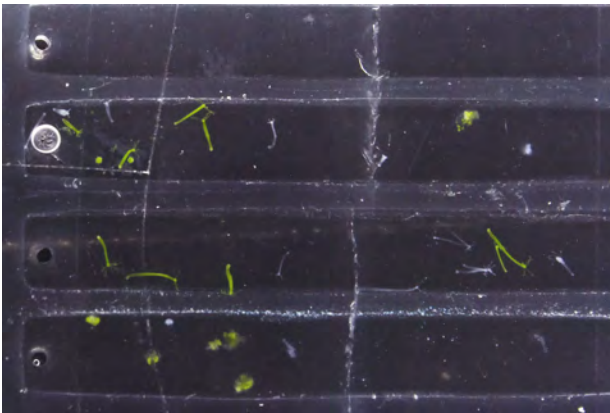
Without Difference Matte   With Difference Matte

Two ways to create a source layer:

A. During the footage collection stage, capture one frame/image **without** any organisms in the frame (ex: empty petri dish)

   • IMPORTANT: this image must be taken in the **exact same position** as the rest of your footage. Everything in the frame must be the same except for the removal of the organisms.

B. Create an artificial source layer in Photoshop (*see next page*)

Creating a source layer in Photoshop:

1. Open Adobe Photoshop

2. File > Scripts > **Load files into Stack…**

3. Click **"Browse…"** and choose 5-10 images from your footage
   - Get a variety from the beginning, middle, and end of your recording
   - If you are using a video file instead of an image sequence, export some images first (*see external link*)

4. Check the boxes for "Attempt to Automatically Align Source Images" and "Create Smart Object"

5. Click OK
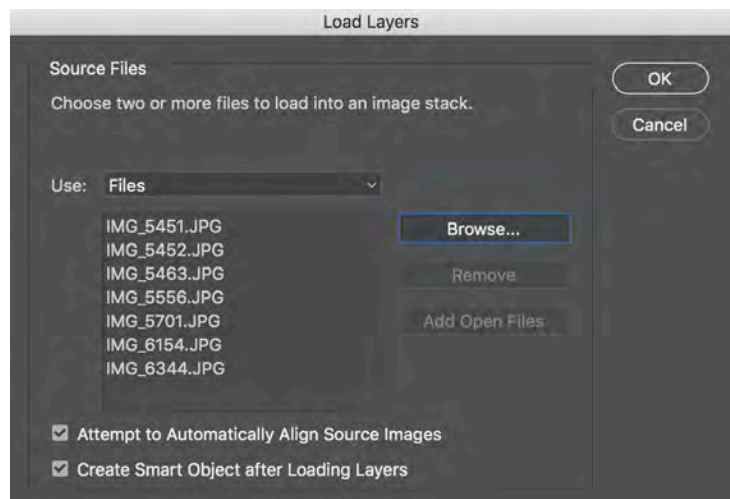
6. Layer > Smart Objects > Stack Mode > **Median**

7. If any hydra remain, use the "Spot Healing Brush Tool"

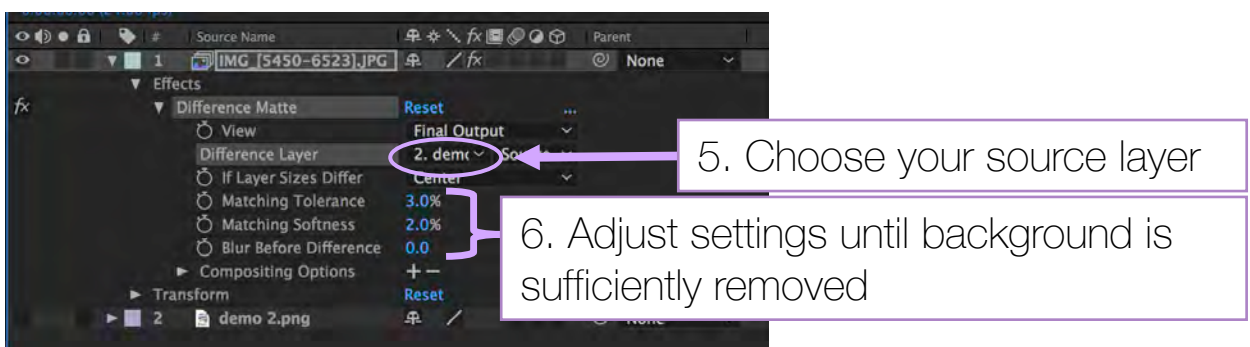8. To export the image, File > Export > **Quick Export as PNG**

Import the source layer

IMPORTANT: make sure any "curves" adjustment is **after** the difference matte effect

1. Import the source layer you created into the Project Panel

2. Click and drag the source layer into the Timeline, underneath your footage (in the example below, my source layer is called "demo 2.png")

Apply the Difference Matte in AE

1. Make sure your footage is selected

2. Click on the Effects Panel > Keying > Difference Matte

3. The effect will appear here

4. Press the eye icon to hide the source layer from view

5. Choose your source layer

6. Adjust settings until background is sufficiently removed
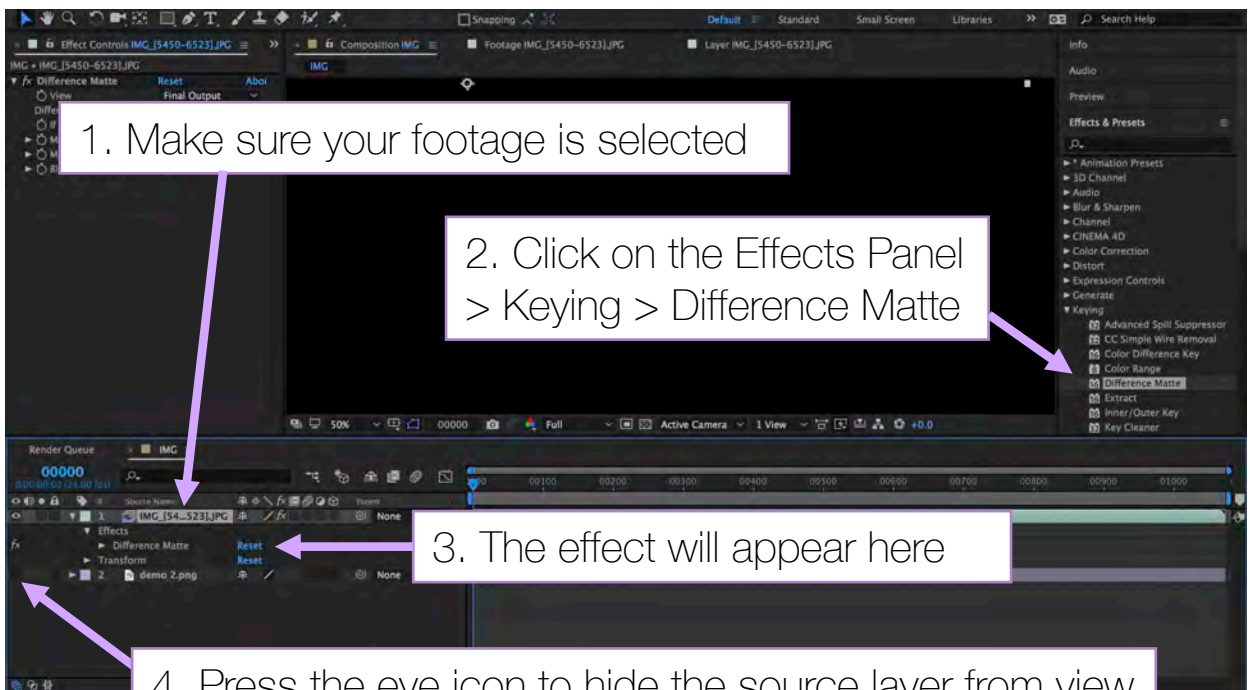
LINEAR COLOR KEY vs. DIFFERENCE MATTE
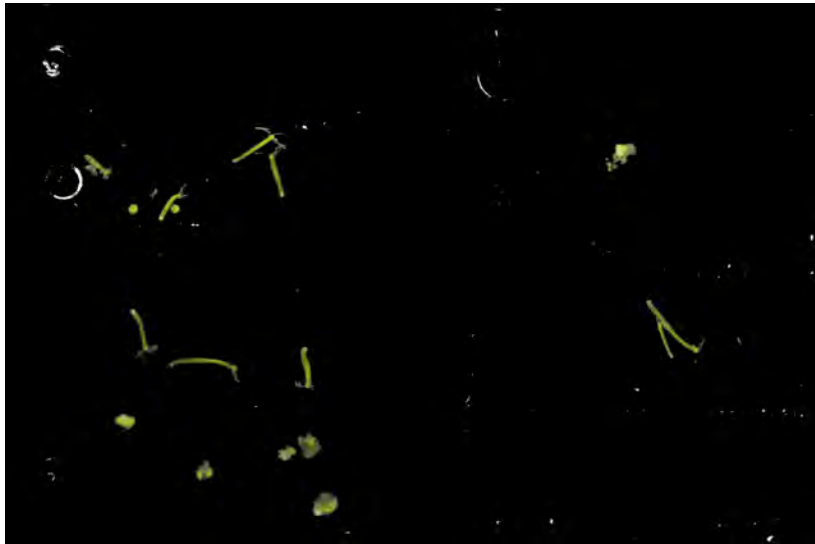
Linear Color Key:

- Clean results

- Easy to use

- Only works when background color **contrasts** with the subject

Transparent hydra shows up with the Difference Matte, but not with the Linear Color key
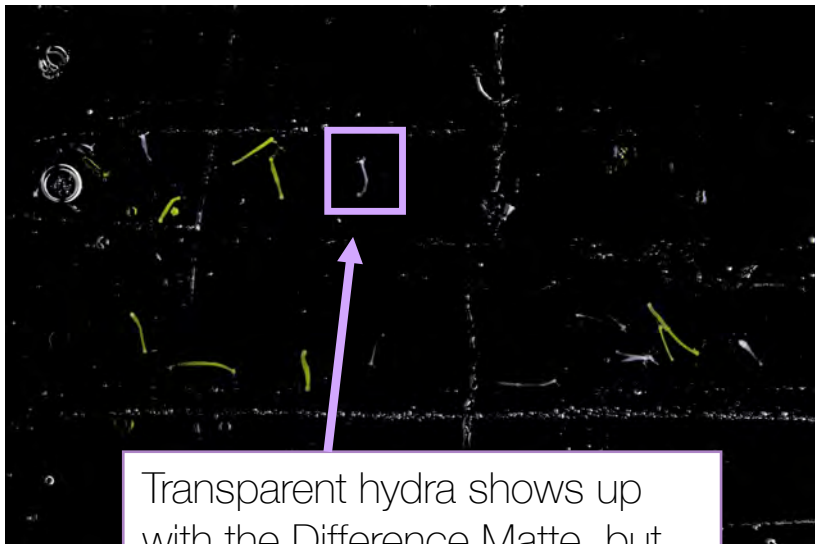
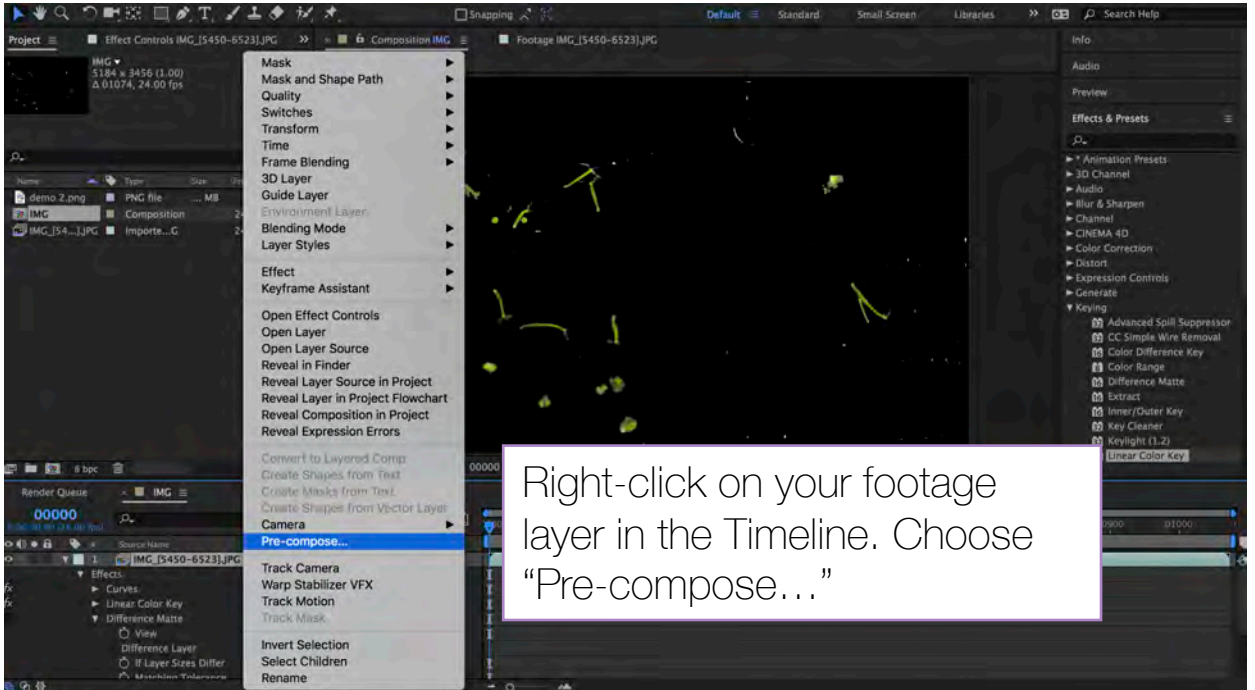## Difference Matte:

- Results vary depending on the quality of the source layer

- Difficult to use

- Can work regardless of background

Use either the Difference Matte or Linear Color Key as desired, but **NOT** both.

FINALIZING FOOTAGE

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Right-click on your footage layer in the Timeline. Choose "Pre-compose…"

Pre-compose

Name → New composition name: Finalized Footage

○ Leave all attributes in 'IMG'
Use this option to create a new intermediate composition with only 'IMG_[5450–6523].JPG', in it. The new composition will become the source to the current layer.

Choose → ● Move all attributes into the new composition
Use this option to place the currently selected layers together into a new intermediate composition.

☐ Adjust composition duration to the time span of the selected layers
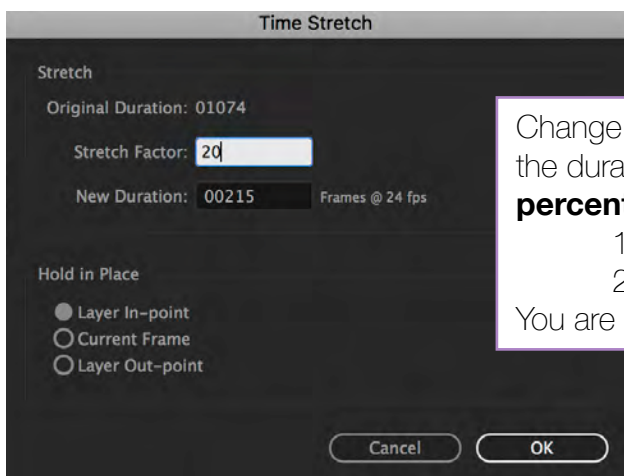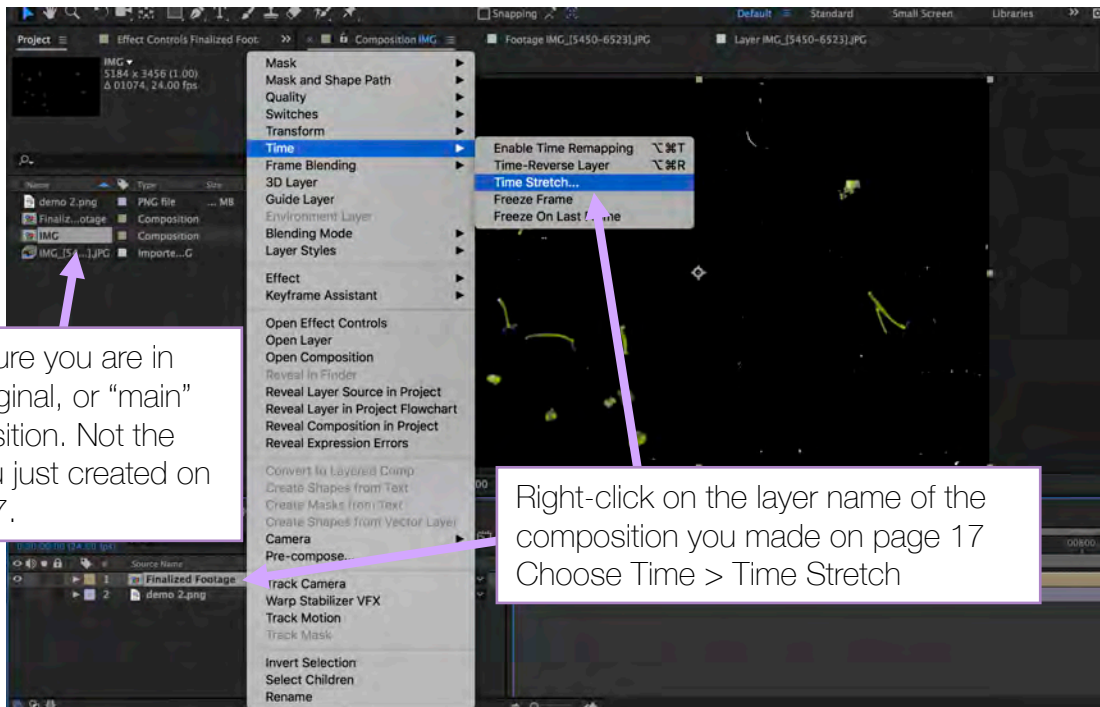
☑ Open New Composition

Cancel   OK

Note: This new composition is contained within your original composition

✓ **Now the footage is ready for motion tracking**   Click OK

TIME STRETCHING

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Optional: Time Stretching

Used for speeding up footage. If your subjects move infrequently or slowly, time stretching might help reduce processing time during the motion tracking. For Matlab, it may also improve accuracy.

Make sure you are in your original, or "main" composition. Not the one you just created on page 17.

Right-click on the layer name of the composition you made on page 17 Choose Time > Time Stretch

Change the "Stretch Factor" to change the duration. Think of the value as a **percentage of original duration.**
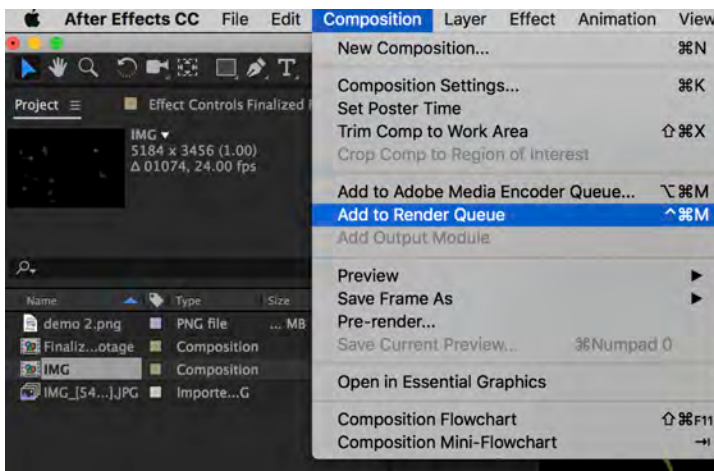100 = 100% (same speed)
20 = 20% (faster than original)
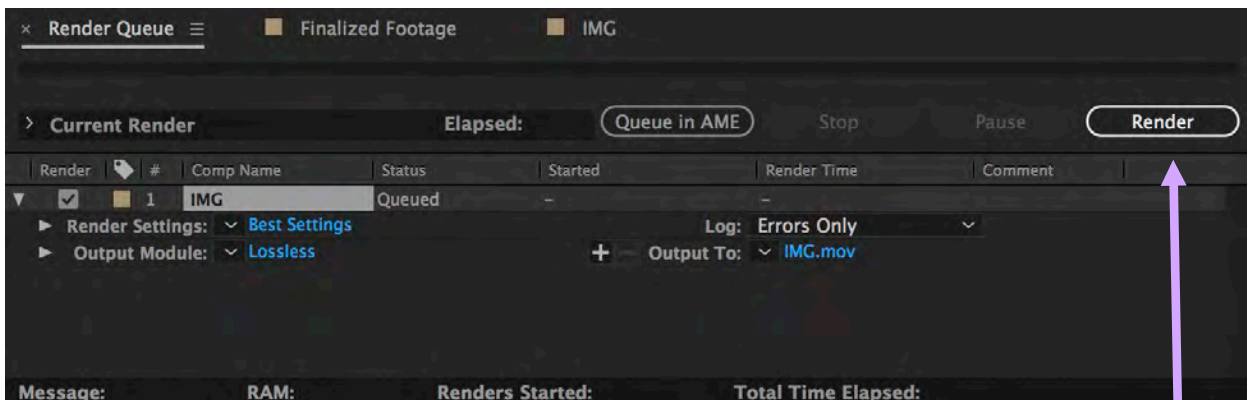You are **dropping** frames to speed up

Optional: Exporting

You can export your adjusted footage to be used in other software. If you are motion tracking in AE, this step is unnecessary.

With your **main** composition selected, choose "Composition" > "Add to Render Queue"

The Timeline will now change into the Render Queue Panel.
Change render settings as needed and choose an output folder.
• You can choose to export as a video file in various formats or as image sequences
Click "Render" to create an exported file.

MOTION TRACKING IN
ADOBE AFTER EFFECTS

SETTING UP

1. Make sure your **main** composition is selected, not the finalized footage you made on pg. 17

2. In the Timeline, also check that the **main** composition tab is selected

3. Finally, make sure that the **layer** with the finalized footage is selected

4. Click on the **"Tracker"** panel

ADDING TRACK POINTS
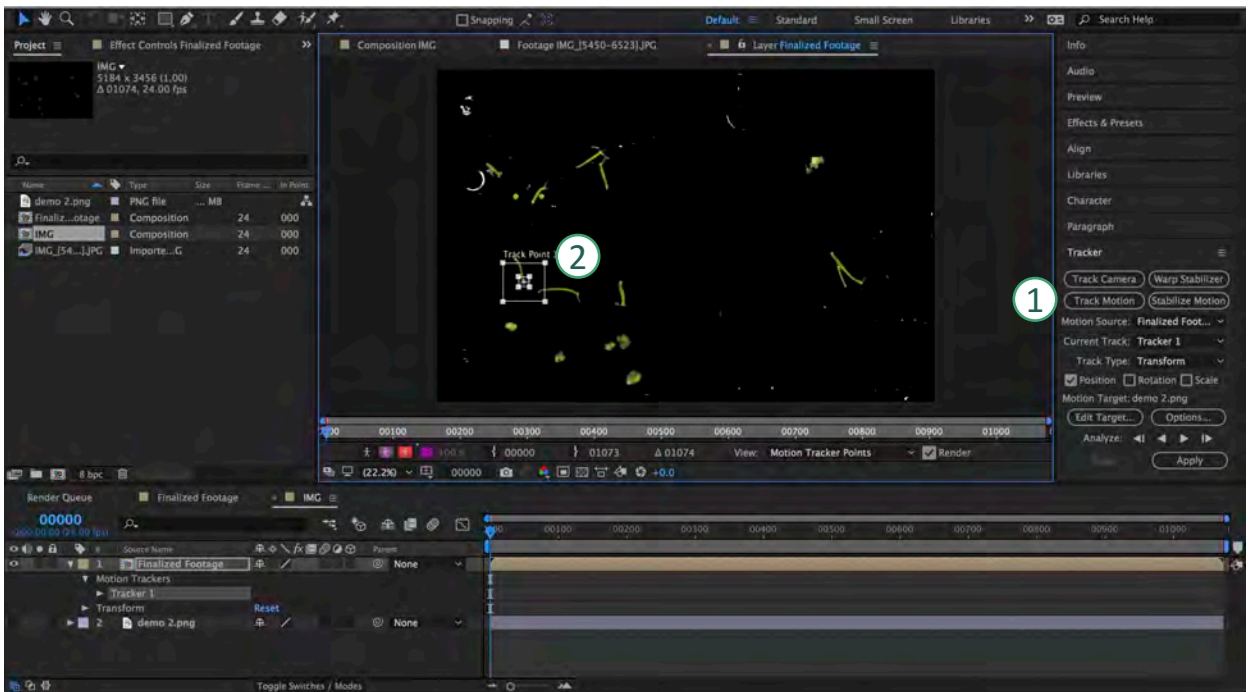
1. Click "Track Motion"

2. A "Track Point" box will appear on the composition panel. **Click & drag** the box over the subject you want to track.

   - When you drag, the box will be temporarily magnified for greater accuracy

   - The inner box tells AE **what to look for** during the tracking

   - The outer box is the **search region** that AE uses to look for your subject

   - The **+** in the middle of box is the "Attach Point." This is the point whose coordinates are recorded. Place the Attach Point over an identifiable part of your subject (i.e. a part of the subject that contrasts with the background)

   - Click and drag the 4 points around the box to resize/reshape the boxes. Both the inner and outer box can be resized.

Troubleshooting

   - If the boxes are not moving together, you may be moving the attach point

   - If you can't see any track point boxes, make sure you are in the correct composition and click on the "Tracker" layer in the Timeline

ADJUSTING THE TRACKER
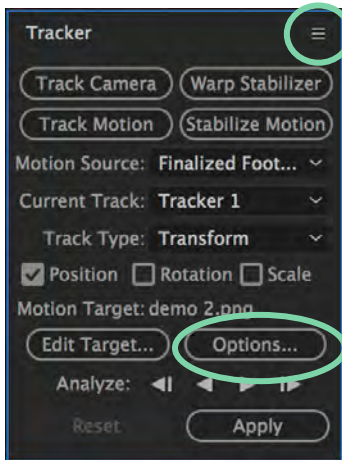
To add more track points, click the ☰ button on the Tracker Panel and choose "New Track Point"

Repeat the same steps as before to position all the new track points

Press the "Options…" button to bring up the options window

Channel: Select **RGB** if the tracked feature is a distinct color. Select **Luminance** if the tracked feature has a different brightness than the surrounding. Select **Saturation** if the tracked feature has a high concentration of color, surrounded by variations of the same color (such as a bright red scarf against a brick wall).

Enhance Before Match: Temporarily blurs or sharpens an image to improve tracking.

Track Fields: Temporarily doubles the frame rate of the composition and interpolates each field to a full frame to track motion in both fields of interlaced video.

Adapt Feature On Every Frame: Causes After Effects to adapt the tracked feature for each frame. The image data that is searched for within the search region is the image data that was within the feature region in the previous frame, rather than the image data that was in the feature region at the beginning of analysis.

If Confidence Is Below: Specifies the action to perform when the Confidence property value is below the percentage value that you specify.

- Select **Continue Tracking** to ignore the Confidence value.

- Select **Extrapolate Motion** to estimate the position of the feature region. Attach-point keyframes aren't created for low-confidence frames, and attach-point keyframes for the low-confidence frames from previous tracks are deleted.

- Select **Adapt Feature** to use the original tracked feature until the confidence level falls below the specified threshold. At that point, After Effects adapts the tracked feature to be the contents of the feature region in the frame preceding the one that has low confidence and continues tracking.
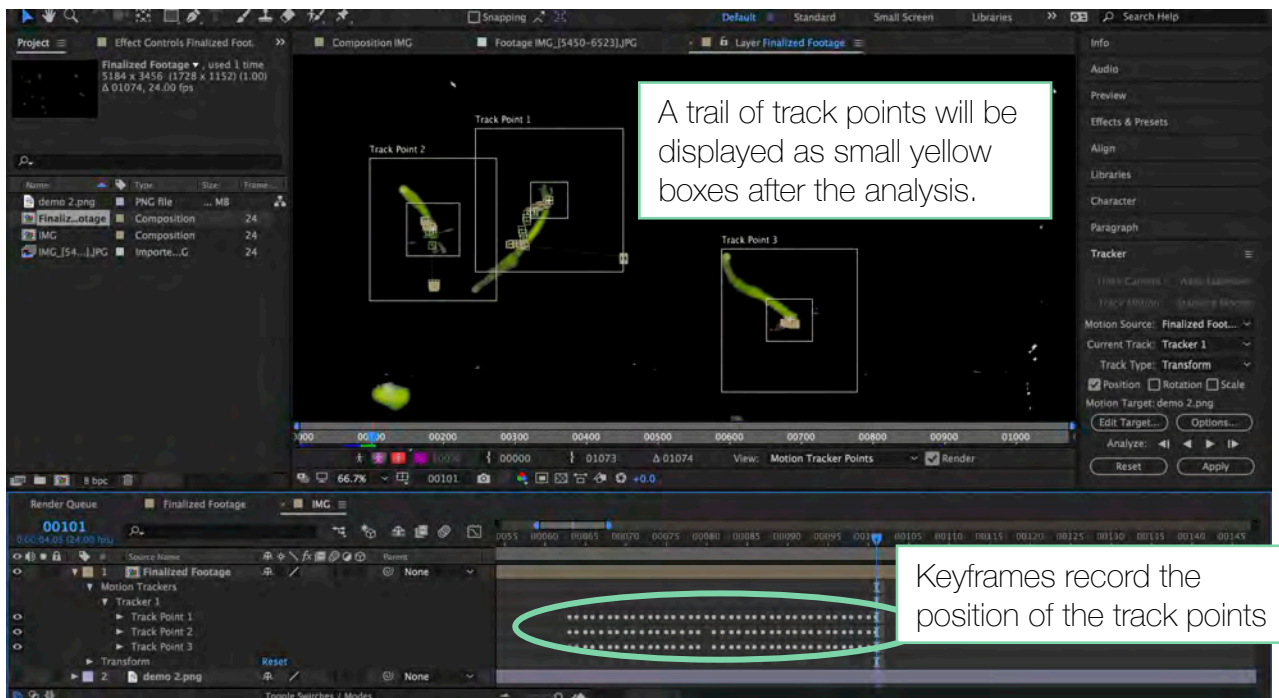
RUNNING THE TRACKER

Press the Analyze buttons on the Tracker Panel to begin the tracking process.
◄I Analyze 1 Frame Backward: Analyze the current frame by moving back one frame
◄ Analyze Backward: Analyze from the current-time indicator backward to the beginning
► Analyze Forward: Analyze from the current-time indicator to the end
I► Analyze 1 Frame Forward: Analyze the current frame by advancing to the next frame

When the analysis is in progress, the Analyze Forward button changes to a Stop button that you can press to stop the analysis at any point.

In this example below, the analysis ran from frame 63 to 101.

A trail of track points will be displayed as small yellow boxes after the analysis.

Keyframes record the position of the track points

! If the track points drift away from the subject during the analysis, you can **stop** the analysis and **manually reposition the track point(s)** and then press the analyze button again. You can move the playhead to find the specific frame from which you must restart the analysis. (i.e. no need to restart from the beginning)

SAVING DATA

To save the results of the tracker, go into the Timeline and select Motion Trackers > Tracker # > Track Point # > **Feature Center**
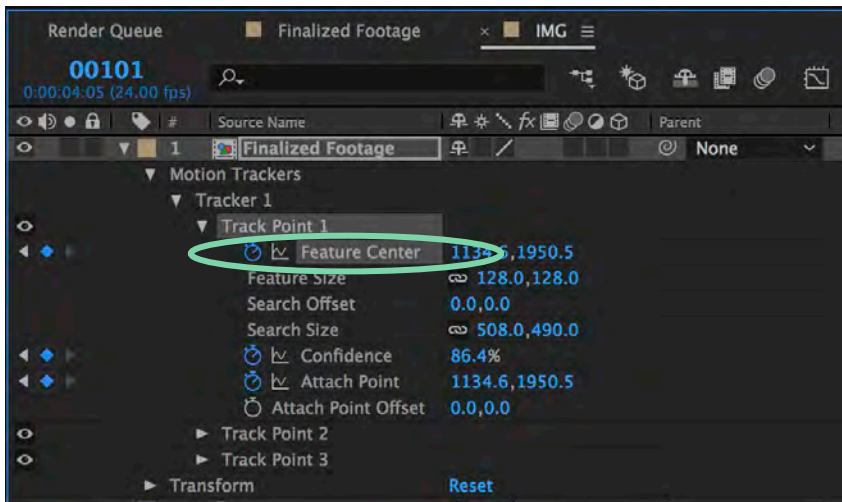
Press Cmd+C to **copy** the position data of the track point. Cmd+V to **paste** the data in any document, such as an excel spreadsheet:

The data from the Feature Center is the pixel coordinates of the Attach Point

Repeat for multiple track points as needed.

TIPS/TROUBLESHOOTING

How big should my Search Region (outer box) be?
The bigger the search region, the longer the processing time.
Also, if the search region covers many subjects that look identical, the tracker might accidently jump from one subject to another.

How do I track the changes in size of my subject?
- If you are tracking a *single* subject, you can check both the "Position" and "Scale" boxes on the Tracker Panel. A second track point with a line connected to the first track point will appear. Position both track points on the same subject (ex: one on the head and one on the tail). Now the *distance* between the two track points will be recorded.
- Alternatively, with multiple subjects, only keep the "Position" box checked and place two track points on each subject. After you copy the Feature Center data onto a spreadsheet, you can calculate the distances between two related track points yourself. Take note of which track point is which!

Can I leave the Analysis running unattended?
It is recommended that you stop the analysis once every 50 frames to check that all the points are still following your subjects. That way, you can reposition them as soon as possible.

How do I reduce the tracking time?
- You can export the video first (see pg. 19) and then import it back, create a new composition with the new video, and then add the track points.
- Reduce the size of the search region and the feature region (both boxes)

The track points do not follow my subjects very well
- Try adjusting the options in the Tracker panel
- Increase the size of the Search Region (the outer box)
- Or re-collect footage at a higher fps

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