An Illustrated, Step by Step Workflow for Digitizing Video Home Systems, Enhancing Their Visual Quality, Placing a Screen-Visible Time Stamp, and Tracking Movement Using Computer Vision Technology[1]

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Abstract: We present an illustrated workflow for digitizing Video Home Systems (VHS) videos, enhancing the visual quality of the digitized videos, placing a screen-visible time stamp, and tracking the movements of imaged entities with computer vision technology. We illustrate this protocol using videos of adult *Drosophila melanogaster* reproductive behavior originally stored as VHS videos. A vast amount of biological information is now potentially easily analyzable by unleashing the awesome power of digital technology.

Key Words: VHS tapes, analog format, digital format, digital video quality improvement, digitally time stamping, computer vision technology, digital automatic tracking, *Drosophila*, behavior, evolution, sexual selection, quantification of observations

Movement is generally considered tantamount with life. When curious about whether something that looks biological is alive, we tend to jolt at the slightest sign of movement realizing that, if it moves, likely, it is alive. Scientific, quantifiable answers to numerous questions pertaining to motion are now more available than ever owing to rapid advances in digital technology. For instance, to ascertain differences between typical and extreme motion of living things (be it of excellence, as in that of sports superstars, or of underachievers), markers are attached to various joints and bony prominences. Thereafter, scientists use computerized optical motion analyses systems identify the markers as they move, creating a detailed report (Maheswaran 2015, O'Sullivan et al. 2014). The machines that garner these data not only have become increasingly miniaturized (as in wearable biometric devices, such as the Fitbit®), abler to learn, faster, more

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affordable, and popular. Also, those devices have become avenues for acquiring self-knowledge, and relatively effortlessly garnering ever more quantifiable data, such as our precise coordinates on Earth (e.g., the location of our vehicle, Humphreys 2012; or participants in a cycling contest or flying airplanes), how many steps we have walked or hours we have slept, etc. (Wolf 2010). Other parties may, at times unbeknown to us, harvest or mine that information creating privacy concerns (Crump 2014, Hyponnen 2013, Kovacs 2012, Spitz 2012). Other applications of tracking technology include serious health conditions (e.g. reduction of geographical disorientation due to Alzheimer's, Shinozuka 2014; detection of autism Klin 2011, or brain injury, Samadani 2015; happiness, Killingworth 2011), gaming, consumer behavior to "increase the shopability of a store" (Burke 2014), design (Oxman 2015), self-driving vehicles, and many others. In organismic and supraorganismic biology, tracking technology connected to satellites or to drones is being used to follow individuals through ecological volumes (Block 2010, Davidson et al. 2014, He et al. 2016, Kays et al. 2015, Koh 2013, Laskin 2013, Ren et al. 2013, Rowcliffe et al. 2016). A large amount of digital position data is currently housed at Movebank (https://www.movebank.org/).

During the mid-1990's, coauthor Santiago-Blay explored issues of speciation through sexual selection. He observed the courtship of different genetic varieties of *Drosophila* flies (Diptera) and, as typically done then, stored the behavioral information in Video Home Systems (VHS) tapes, an analog storage system whose heyday in the USA, was, approximately, 1970-2005. This presented him with the challenge of quantifying the documented observations in detail through the flies' courtship. Some twenty years later, he joined forces with several colleagues (coauthors) who have the motivation and the expertise in computer sciences to tackle the practical biological research question, can VHS videos be rendered suitable for rapid and accurate quantification of biological phenomena? The answer is "yes" and this paper explains how we did it by providing an illustrated workflow using examples of *Drosophila* courtship originally taken in the mid -1990's.

How to rapidly quantify the information stored in the VHS tapes? As VHS format uses analog signals to store and transmit data, the very nature of these tapes makes them difficult to readily analyze in the digital world of computers. The decline in analog's popularity (approximately 1995-to the present), along with a newfound appreciation for digital data archiving, has spurred efforts to convert information previously stored in analog format into digital, which can be done with relative ease and great accuracy. Recently, software has become more available to assist in converting analog signals into digital and further enhance their visual quality and add a digital time stamp. Furthermore, computer vision technology software packages, such as *OpenCV* (2016); *ImageJ* ([2016], Rasband 1997-2015, Schneider 2012, Abramoff et al. 2004), with the *Mtrack2* plugin (Stuurman 2003, Klopfenstein, and Vale 2004)]; *WINanalyze* (2016); see also

Parker et al. (2015), have become available. Digital files are easier to edit, share, and ultimately provide users with a substantially higher video quality. Once in digital format, the information becomes available for longer-term storage and computer-based analyses.

One example of such computer-based analysis software is *OpenCV*, an image processing software package that uses computer vision enhanced with various functions. Two of those broad category functions are object detection and a video analysis module. Using software written by coauthor Caprio (available on https://blaypublishers.com/2016/11/22/opencv-files-of-santiago-blay-et-al/), we demonstrate that it is possible to analyze digitized, computer enhanced, and time-stamped behavioral data formerly stored in VHS tapes, track the path of the objects of interest and correct errors in the output the software may have generated. Herein, we explain how, in a step by step fashion. We hope this will help likely users extract and analyze data stored in VHS.

Herein, we provide a workflow and examples to digitize, improve the visual quality, time-stamp, and track movement originally captured in VHS tapes (Figure 1). To facilitate learning and the implementation of the technology, we illustrate as many steps as possible. We realize that the reader may choose to acquire other software, yet the steps herein illustrated will give the reader a fairly good sense of what is involved in the process.

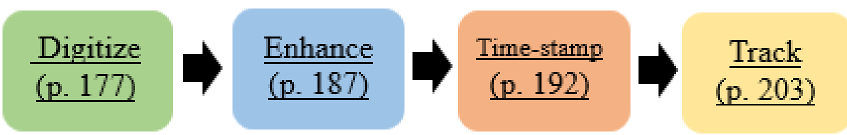

Figure 1. Workflow for Digitizing Video Home Systems (VHS), Enhancing the Visual Quality of the Digitized Video, Placing a Screen-Visible Time Stamp, and Tracking Moving Entities Using Digital Computer Vision Technology. To facilitate mobility between sections, page numbers have been inserted under each header.

Methods

The behaviors were captured by coauthor JASB in the mid 1990's using a dissecting photomicroscope connected to an analog video camera. Virgin flies, *Drosophila melanogaster* Meigen, 1830 or, less frequently, *D. simulans* Sturtevant, 1919, from laboratory colonies, generally 3-7 days young, were used for the study. Two or three (depending on the research goals of the test), *Drosophila* flies were placed in a small (approximately 8 mm inner diameter) empty shell vial plugged with cotton fibers. Two shell vials containing virgin flies and positioned next to each other were videotaped simultaneously to double the amount of information captured per videotaping unit time. Shortly after the beginning of a videotaping session, a small metric ruler was inserted in the video camera field of view for calibration purposes (Figure 2). At the time of videotaping, coauthor JASB did not know how felicitous that decision was as the little ruler would assist in the eventual digital estimation of *x* and *y* positions of the flies. The flies were videotaped until shortly after the last remaining virgin female in a vial mated. As in commonly done in cinematography, we refer to each of the videotaped portions as a "take". Although the videos were qualitatively analyzed (JASB unpublished data), Santiago-Blay and coworkers began digitizing the tapes in 2015 with the long-term goal of quantifying the behaviors. Some 150-200 hours of videotaped courtship from approximately1000 flies have been now digitized.

Figure 2. A digitized (and unimproved) VHS showing six *Drosophila melanogaster* flies, three flies per vial. Note the ruler eventually used to generate x and y coordinates. Compare quality of this image with that on Figure 12.

Digitizing analog VHS videos using Elgato Video Capture software[7]

Surprisingly, the VHS videos remained in excellent condition after 20 years inside cardboard boxes located in unspecialized storage facilities. Each video was reviewed in a VHS cassette recorder and the length of each take rapidly noted using the fast forward control. Takes were digitized using the *Elgato Video Capture* software (https://www.elgato.com/en/video-capture, San Francisco, California, USA; cost approximately 100 US Dollars, excluding taxes and other charges)[8]. Numerous other software packages are available (see Table 1). The *Elgato Video Capture* software was chosen for its ease of use, going directly from VHS to digital, without intermediate steps.

Below, we show a series of screen shots (Figures 3-11) illustrating the steps we followed to digitize an analog video stored in VHS format. Sometimes, takes longer than 45 minutes crashed our computer, necessitating a second (or, rarely, a third) digitizing attempt.

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[7] The video capture software options are very large as there are a variety of quality devices to capture the output of an analog VHS player and convert it to digital PC input. There are also many different software options available. The different variations of these products are in most cases of good quality and are easy to use, but would not be immediately recognized by the typical consumer. Additionally, there are options for all major operating systems. A range of video capture products available for sale can be seen on newegg.com, a popular computer hardware vendor. The ranges of complexity and price are rather wide. Other mainstream devices might come from Hauppauge or Blackmagic. We consider that *Elgato* is probably one of the easiest and most used option, and it is a complete bundle.

[8] As the digitizing time is 1:1 with respect to the VHS time, a small, battery-operated visual alarm was set to blink (go off) shortly before the end of the take. The visual alarm alerted us to prepare to stop digitizing.

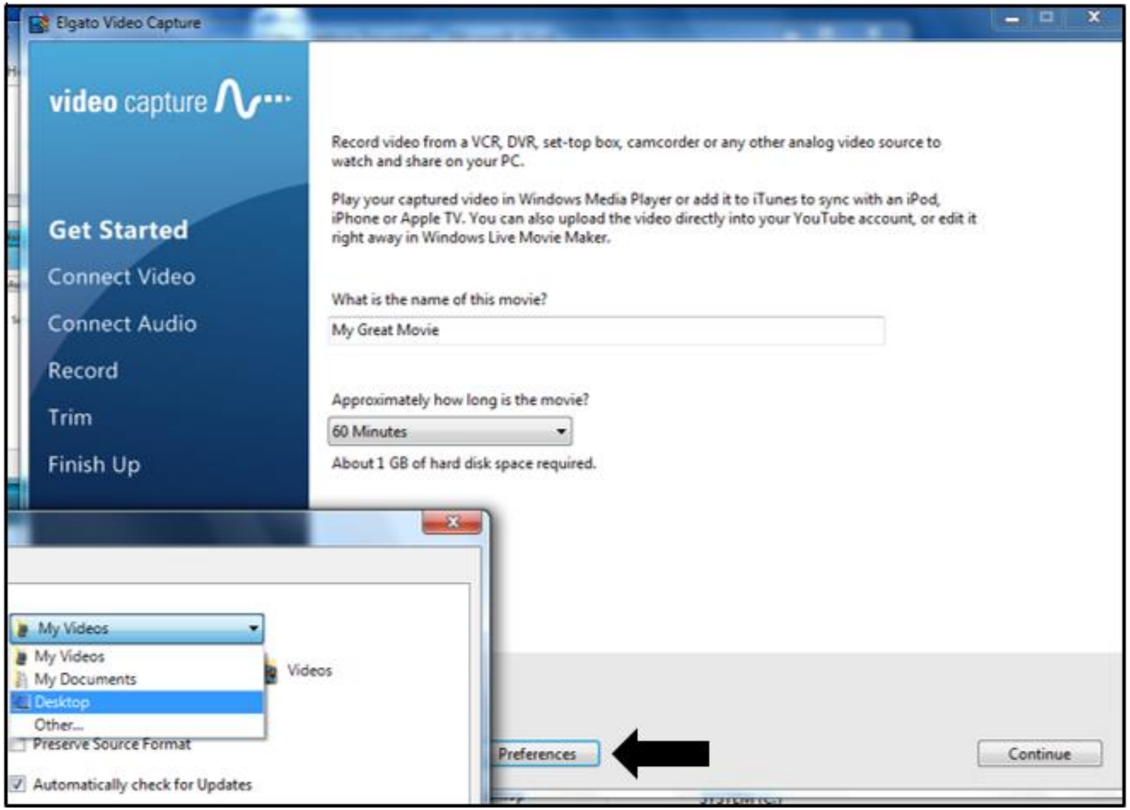


Figure 3. The first time the *Elgato Video Capture* software is used, the user needs to decide where are the digitized videos going to be stored by navigating on the **Preferences** key (arrow). In this case, the user has decided that the videos will be stored in a Folder called, My Videos. This *Elgato Video Capture* screen is seen in most interactions between user and program. Images 3-11 are reproduced with permission from *Elgato Systems*.

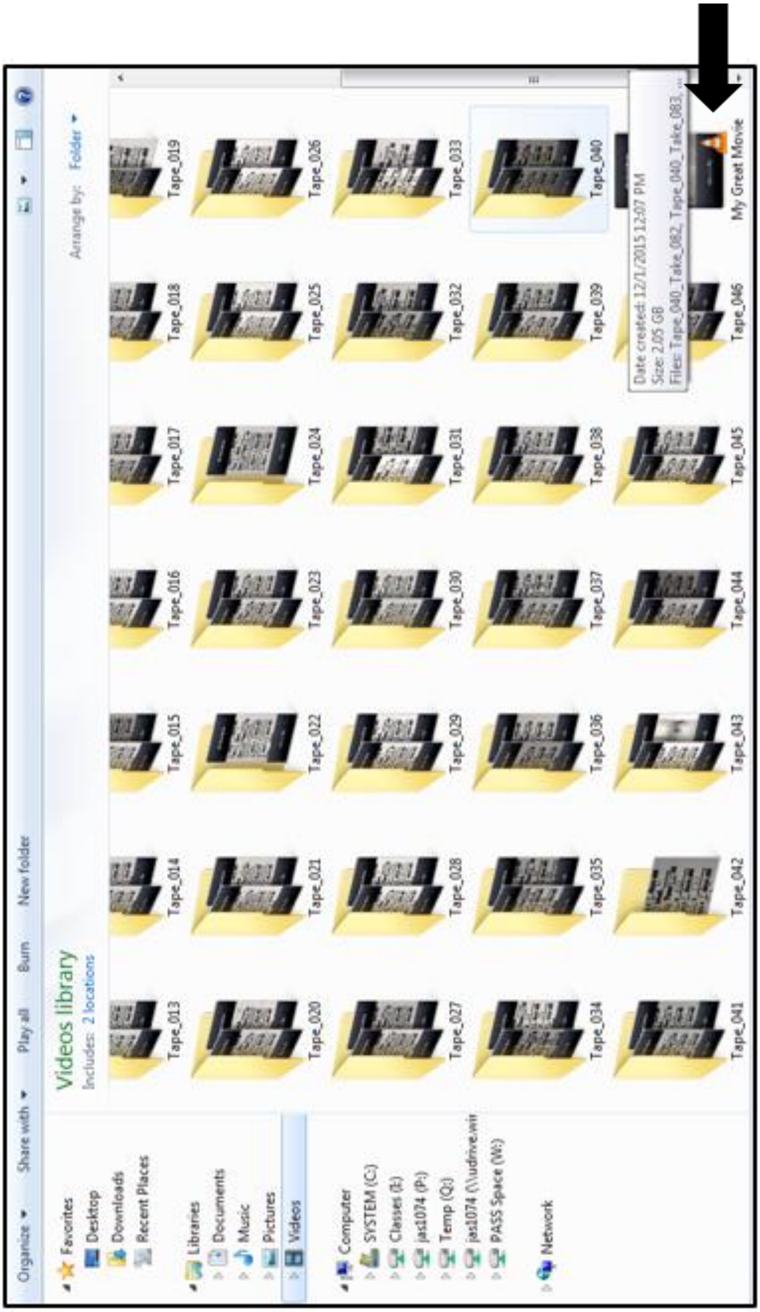


Figure 4. As new takes (e.g., "My Great Video" - arrow) are digitized, they will be stored in My Videos. Thereafter, the user can rename the video file, then drag and drop the video into the appropriate Tape_Number folder.

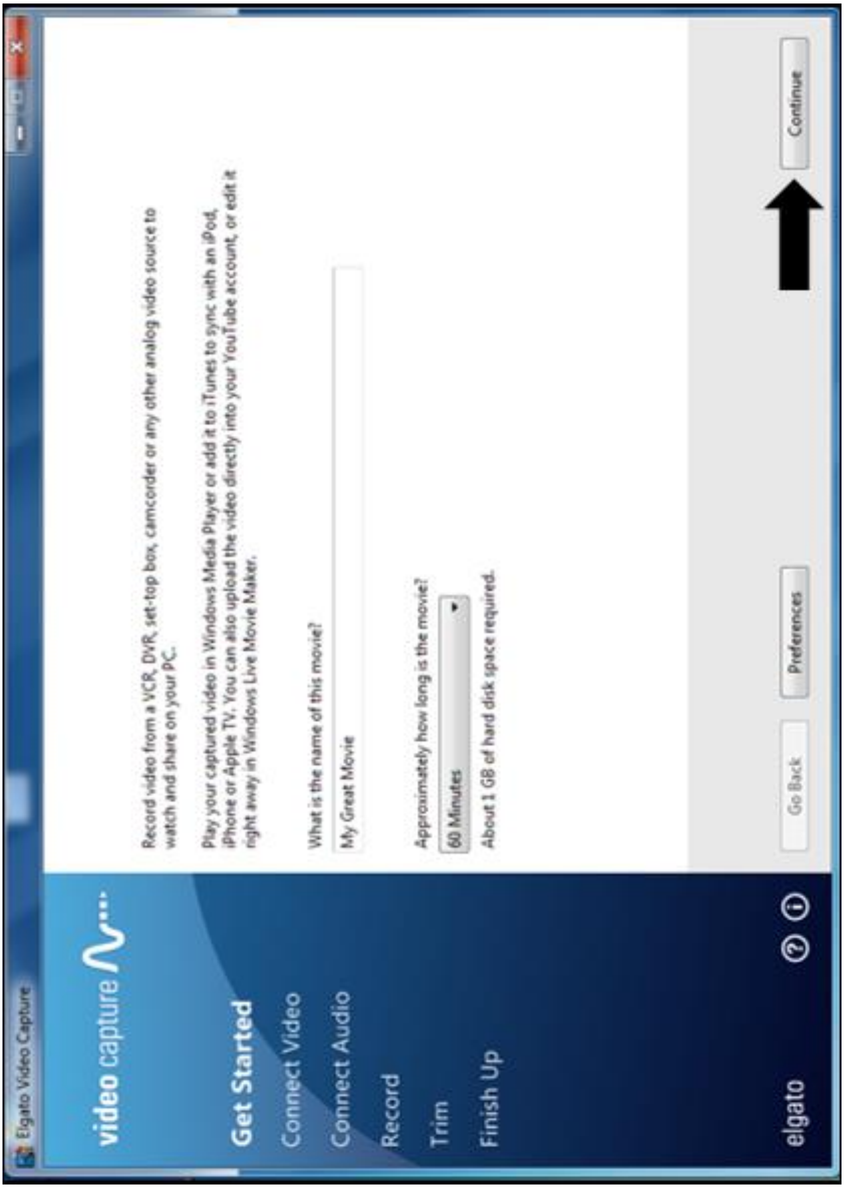


Figure 5. To begin digitization of a take, insert the tape in the VCR (Videocassette Recorder) and press the **Continue** key (arrowhead).

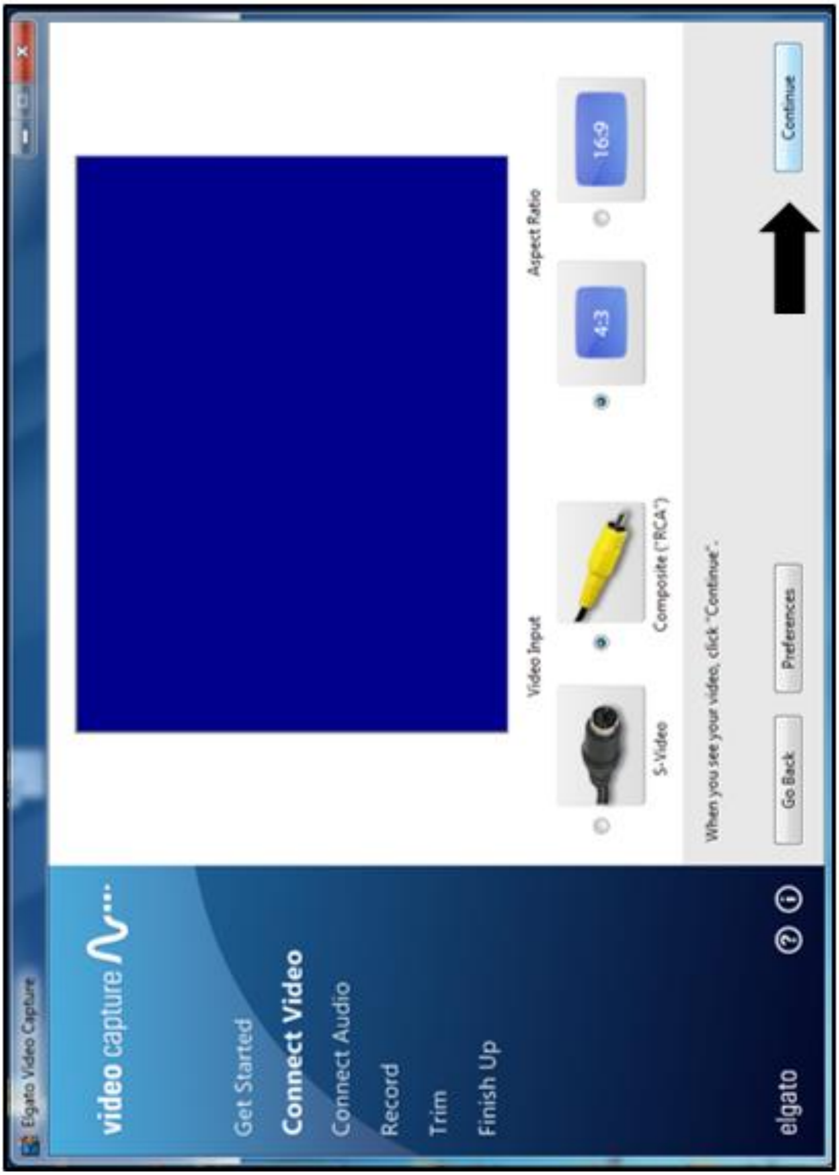


Figure 6. Press the **Continue** key (arrow) on the VCR. This will cause video to begin playing in the *Elgato Video Capture* software screen.

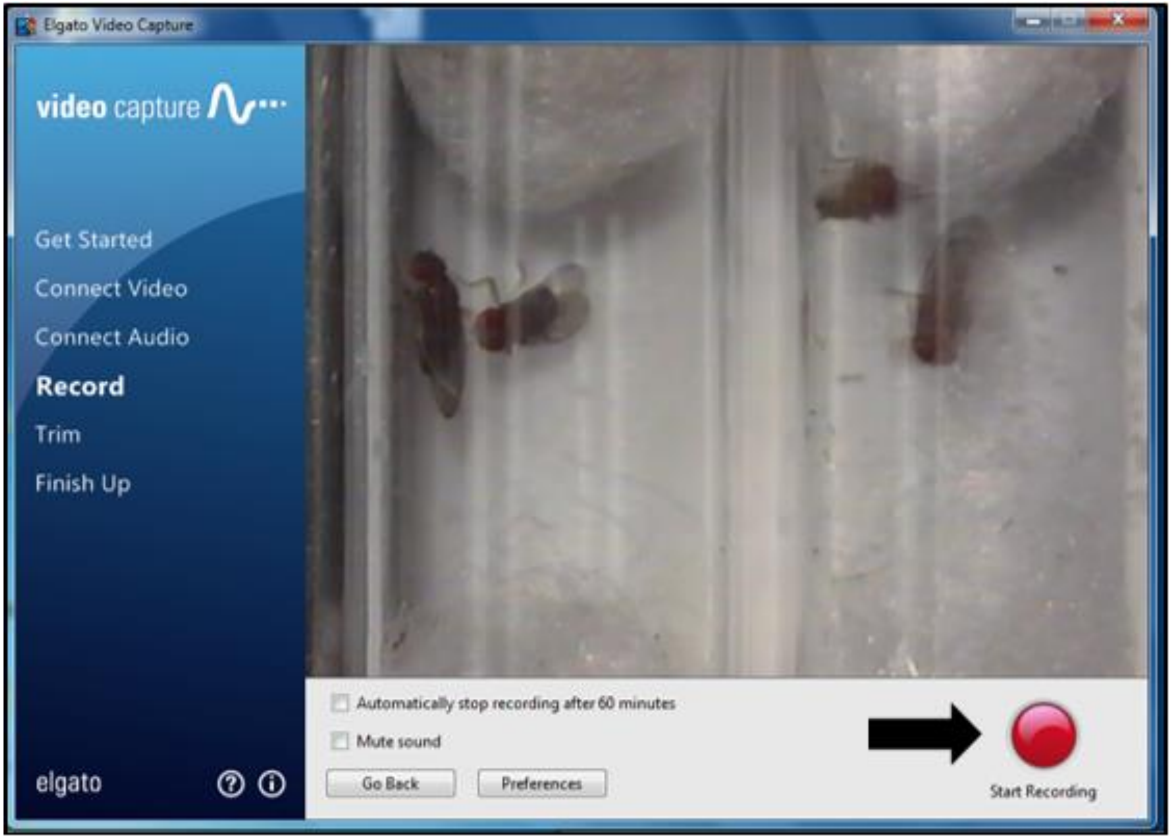


Figure 7. Press the red **Start Recording** button (arrow). This will cause the *Elgato Video Capture* software to begin digitizing the video. "Stop Recording" will replace the bottom "Start Recording" indication once the red record button is pressed (see Figure 8).

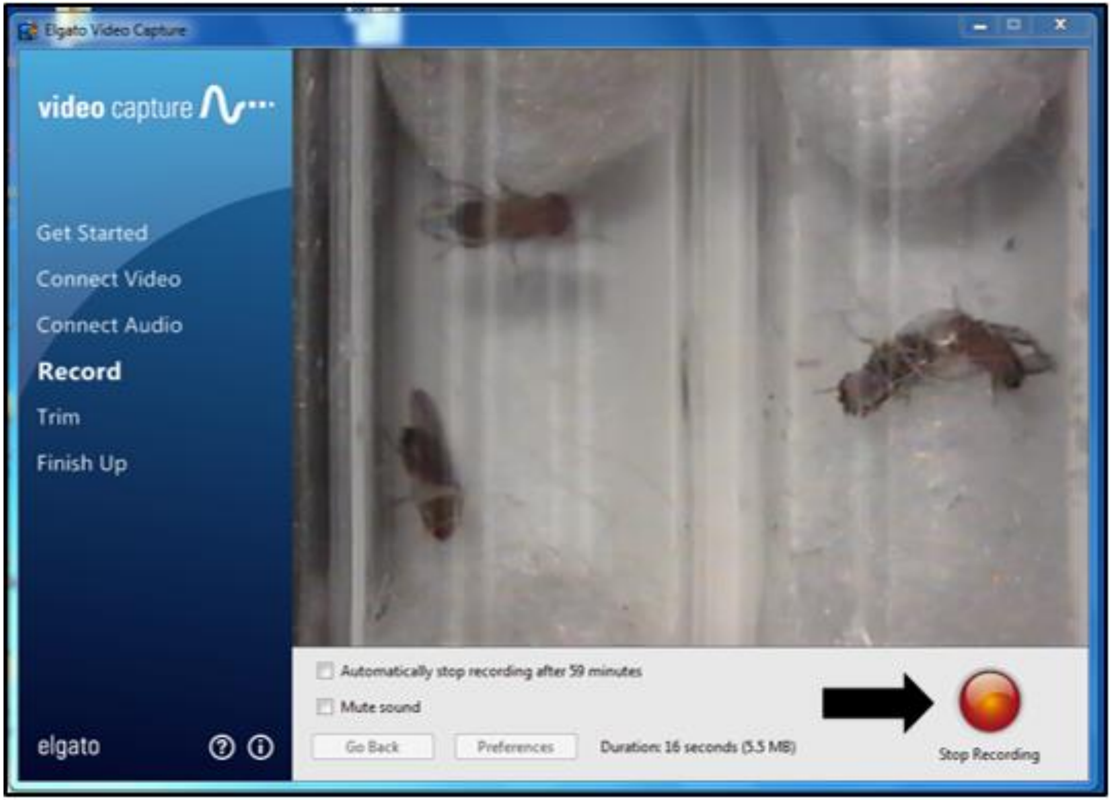


Figure 8. When the video take comes to an end, press the **Stop Recording** button (arrow). This will cause the *Elgato Video Capture* software to stop digitizing the video.

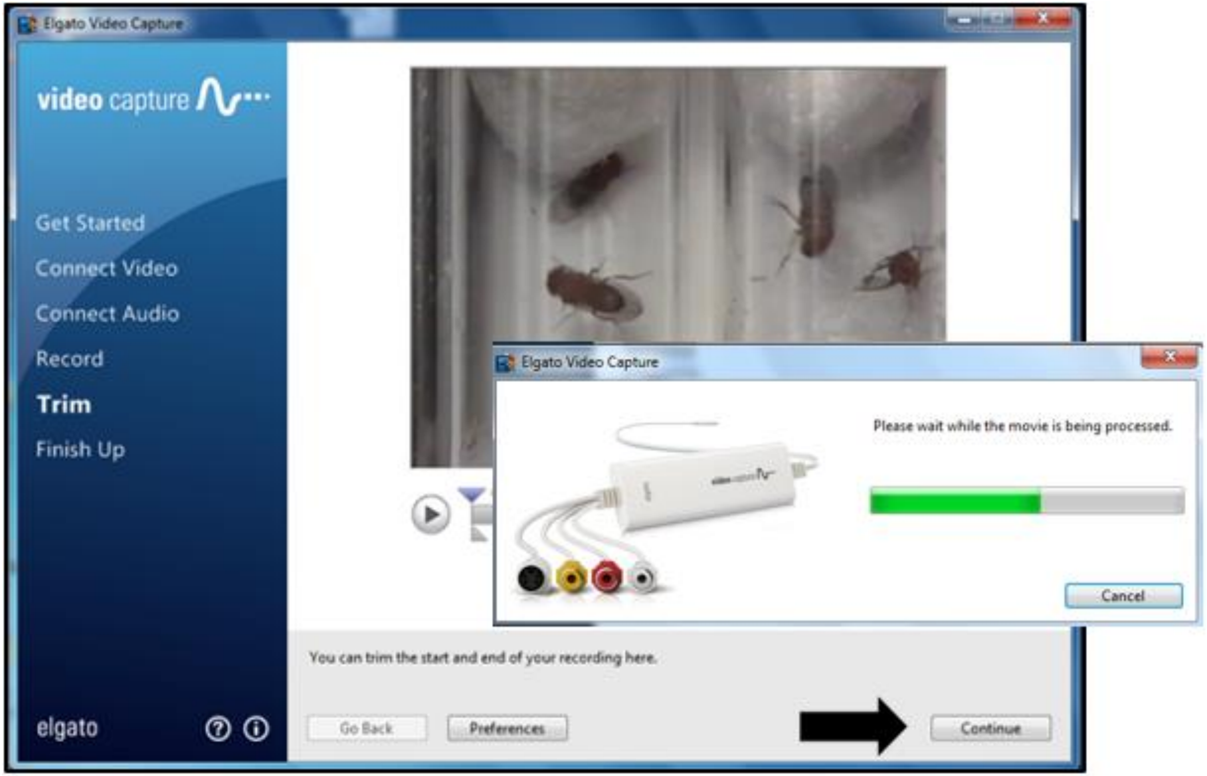


Figure 9. To archive the digitized video, press the **Continue** key (arrow). A green horizontal bar will indicate progress of archiving. Two screen images - the larger image is the typical *Elgato Video Capture* image; the smaller is the interactive message from the digitizing program to the user - have been combined into one.

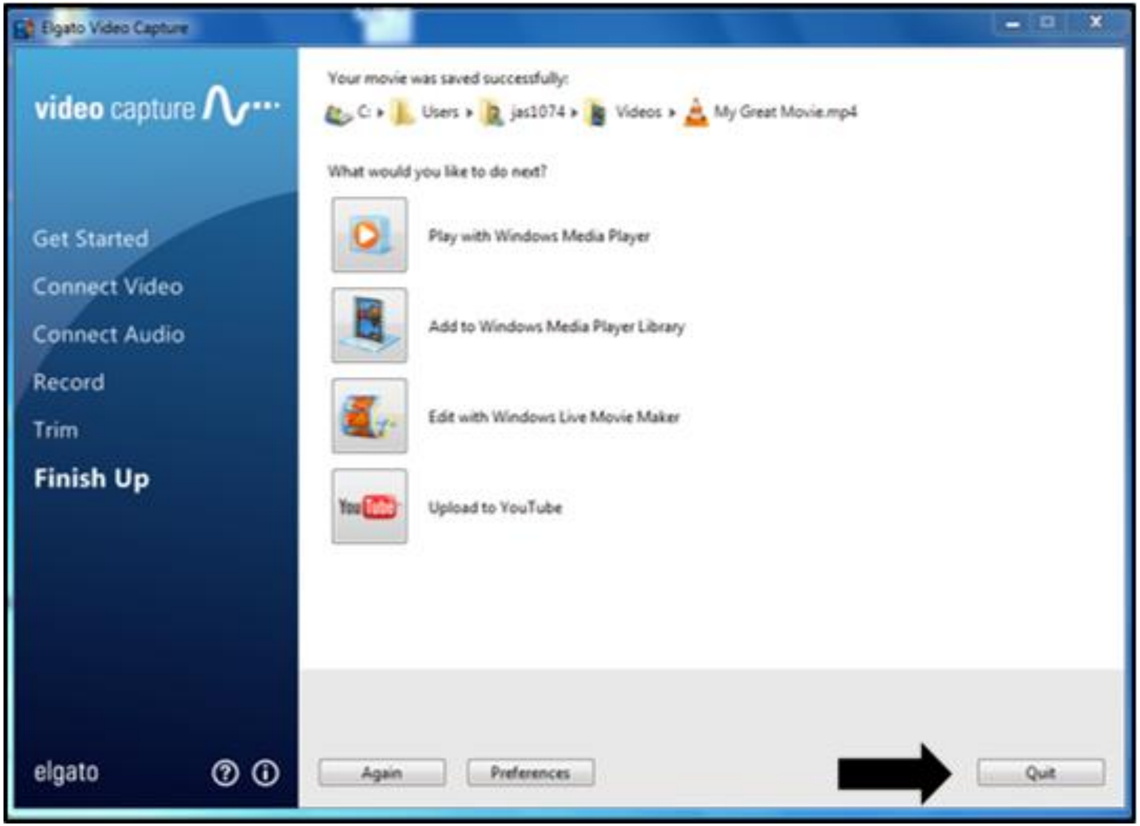


Figure 10. Once the horizontal green bar (Figure 9) reaches the right side, indicating that the archiving of the digitized video is complete, press the **Quit** key (arrow). This will automatically place the video in the place the user designated as place holder for the videos (see Figures 3-4).

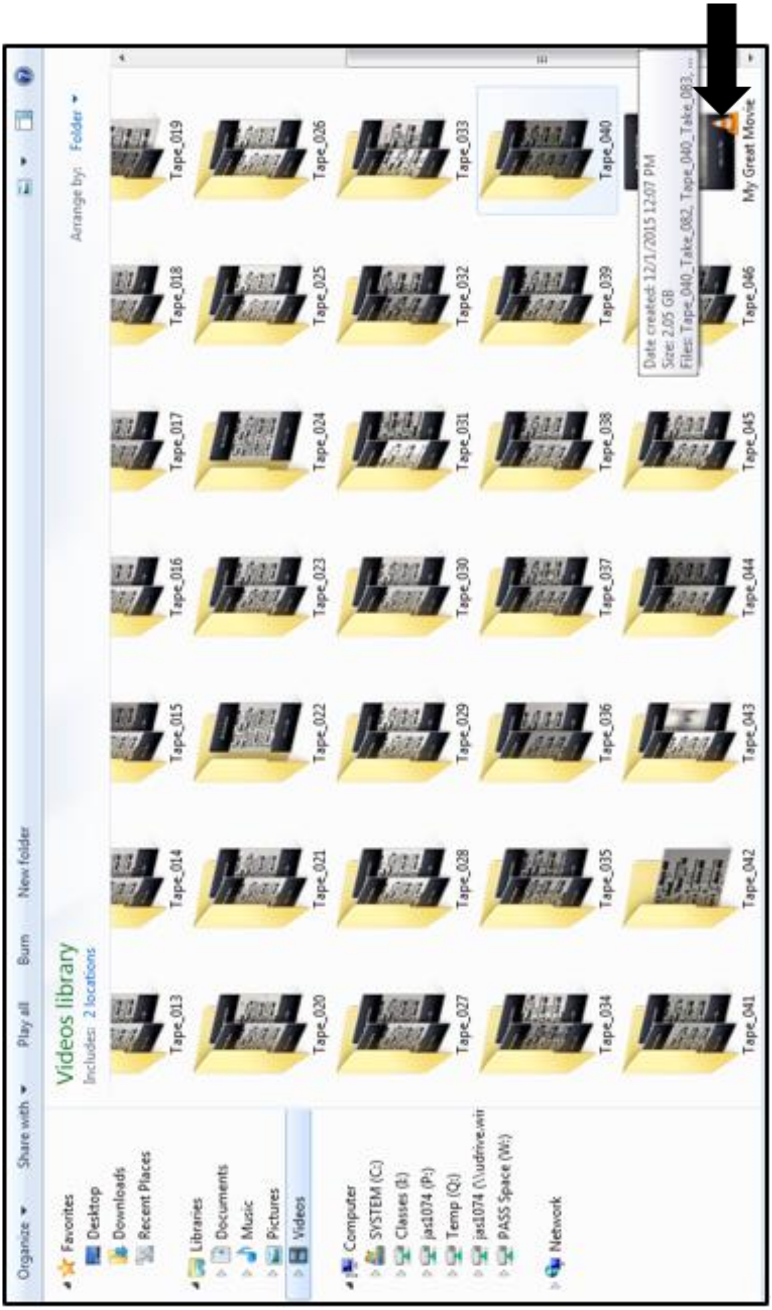


Figure 11. As each new takes is digitized, it is stored in a file entitled “My Great Movie” (arrow) from which the user may want to relabel the video file and move it into the appropriate Tape_Number folder and sub-folder (e.g. Tape_040_Take_082).

Examples of software available to digitize, video enhanced, and time-stamp are provided in Table 1.

Table 1. Examples of available software to digitize, video enhance, and time-stamp videos. The options we used are **boldfaced**. The mention of any of these products does not imply endorsement; only that they exist.

	Windows	**MacOS**
Digitizing	***Elgato Video Capture***	***Elgato Video Capture***
Video Enhancement	*Adobe Premier, Windows Movie Maker, Corel VideoStudio*	***iMovie,*** *Adobe Premier, Final Cut Pro, Corel VideoStudio, Pinnacle Studio, Nero Video*
Time-stamping	***DaVinci Resolve***	***DaVinci Resolve***

Video quality enhancements using iMovie for Macintosh

Figures 12 - 15 illustrate the steps we followed to enhance the visual quality of the digitized videos using *iMovie* for Macintosh (version 10.1.1, Apple, Cupertino, California, USA). These videos (or "takes") had been digitized with a computer that uses Windows operating system. Cost of *iMovie?* The image quality is enhanced dramatically after a few keystrokes (compare Figures 2 and 15, two typical screen shots representing the original digitized and the improved videos).

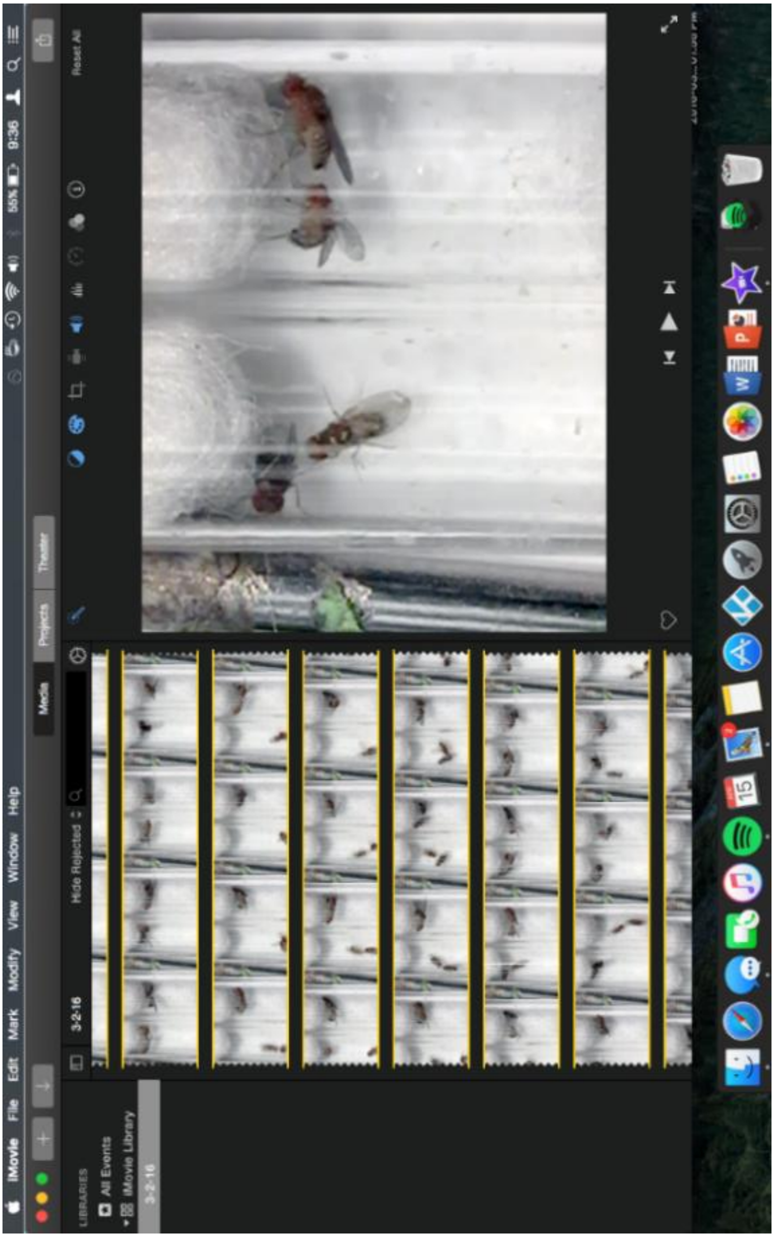


Figure 12. Open a digitized take file with *iMovie* (http://www.apple.com/mac/imovie/). Note enhanced clarity of the image as compared to a typical image, such as that displayed in Figure 2.

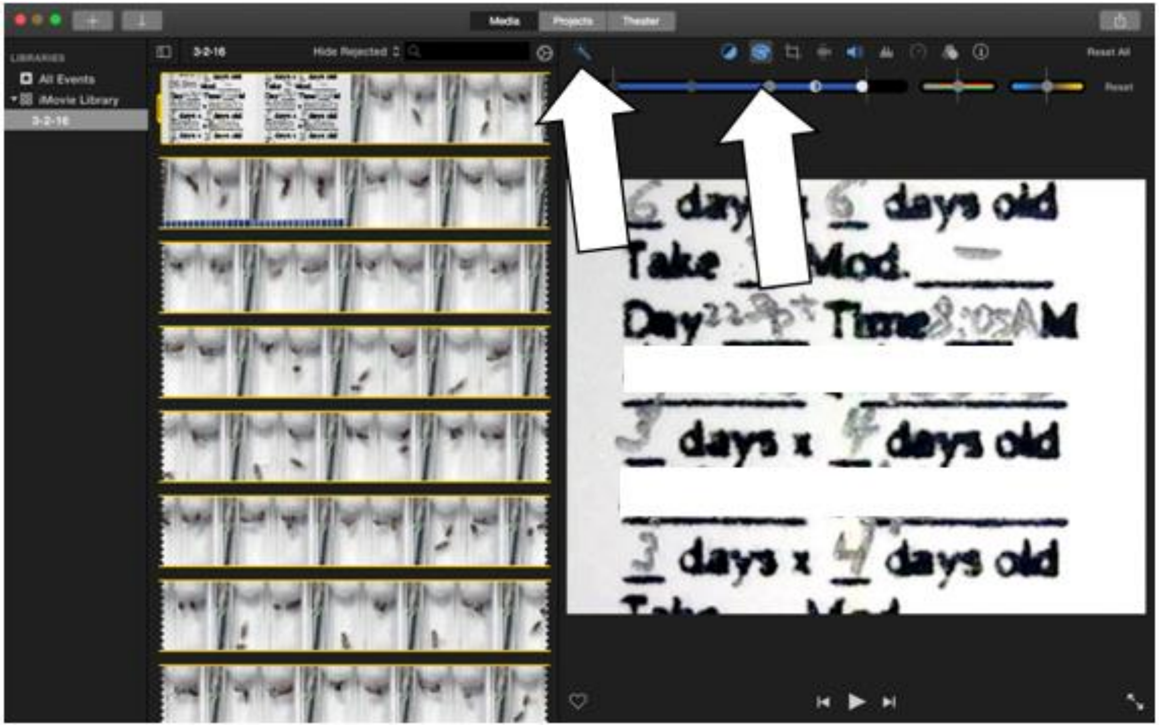


Figure 13. Click the wand under “Projects” (left arrow) to automatically improve the video quality. Clicking the “color correction” pallet on the top right (right arrow) and adjusting the slider will give more control over color adjustments. Because author JASB included the label describing what was about to be videotaped and added a small ruler calibrated in metric to each video, the tracking software can generate x and y coordinates, and time in a spreadsheet like format. The genetic makeup of the flies being tested has been edited out as that work remains unpublished. Additional tapes taken by a predecessor of JASB and made available to him with permission lacked identifying information for the

Figure 14. To export an adjusted video, click the "share" button in the top right (top arrow), then choose "file" (bottom arrow).

Figure 15. Name the file and choose from various video settings (resolution, quality, compression speed or quality, etc.). Click "Next" and choose an export destination on the computer (specific folder, external hard drive, etc.). The information covered by the white rectangles includes the name of the lines used in these experiments. As those data remain unpublished, author JASB prefers to keep it undisclosed at the moment.

Inserting a time stamp to digitized, time-stamped videos

Time-stamp

Figures 16 – 26 are an illustrated step-by-step guide for inserting a time stamp on a digitized video using *DaVinci Resolve* 12.5 for Macintosh (Blackmagic Design, https://www.blackmagicdesign.com/company), a free downloadable application ("app") for computers running on the Macintosh Operating System (Mac OS X) or on Windows available from the Blackmagic Design website, https://www.blackmagicdesign.com/products/davinciresolve. For expediency, it may be beneficial to create a shortcut, or bookmark, to DaVinci Resolve on the reader's device.

DaVinci Resolve can process multiple videos and export them individually. The directions for adding the time stamp explain the process. Two videos are intentionally used in the example herein given to show how multiple videos can be processed simultaneously. According to Blackmagic Design, essentially the software can deliver an unlimited amount of videos at the same time because it is all dependent on how many videos the user puts in the user's timeline. As long as the user delivers individual source clips, the user will be able to process as many clips s/he wants "at the same time".

Open *DaVinci Resolve* by clicking on the white trifoliate icon on a maroon background (Figure 16). The pale blue folder, DaVinci Resolve is where time-stamped videos are placed.

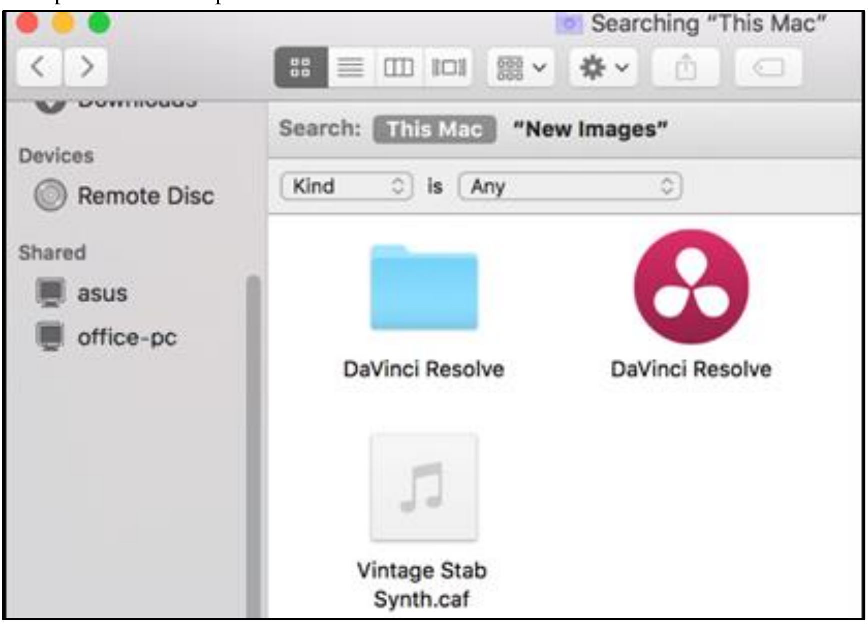


Figure 16. Home screen of DaVinci Resolve.

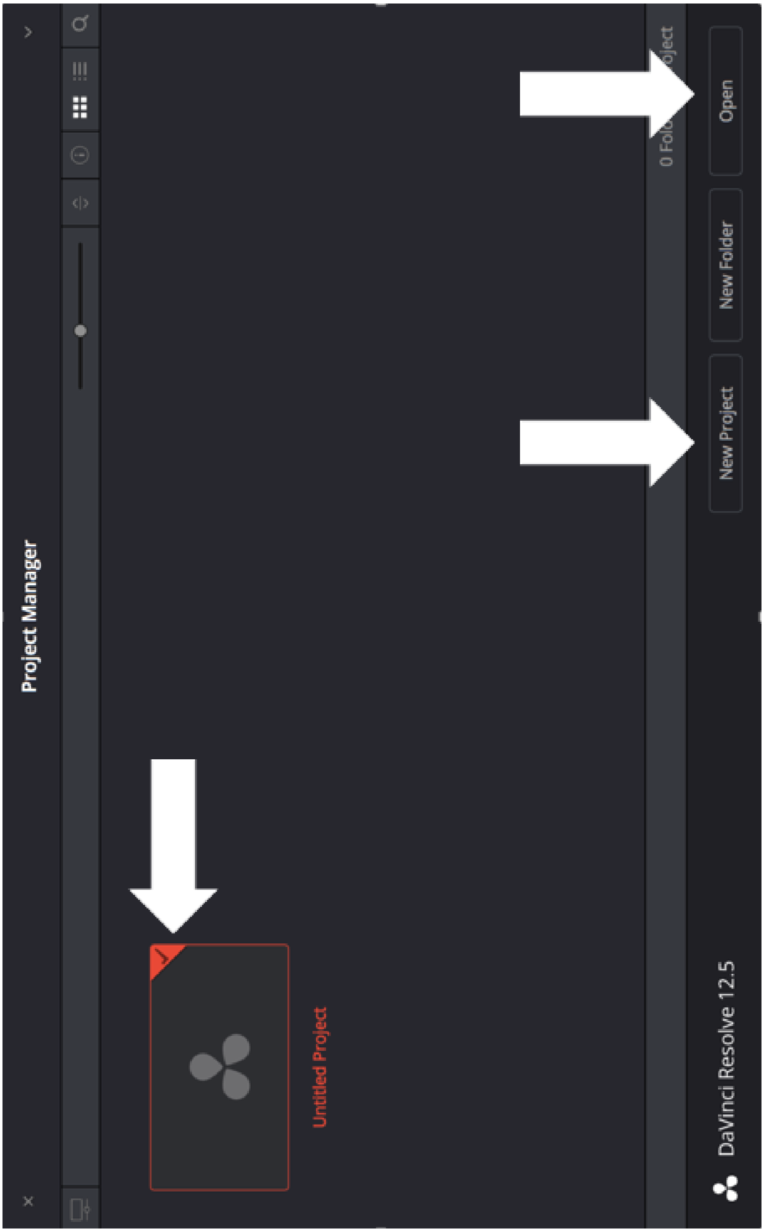


Figure 17. Click "New Project" (lower left arrow) and create a name for the project (e.g. Untitled Project) in the window that appears immediately after pressing "New Project". Make sure the newly named project is selected (red check mark on the upper right hand corner of the file), and click "Open"" (lower right arrow).

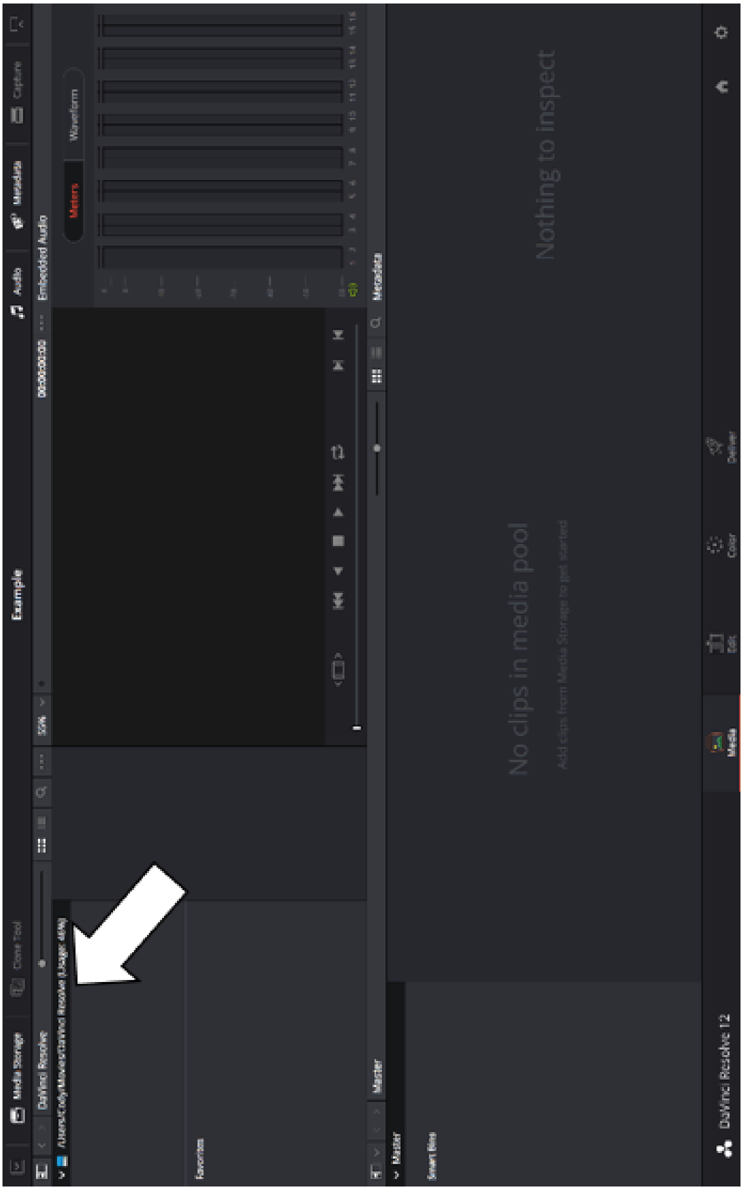


Figure 18. The previous step will create a new folder called "Davinci Resolve" in the user home folder under "Movies" (e.g. /Users/pennstate/Movies/DaVinci Resolve). The exact location of the source video(s) to be time-stamped will be shown in the top left of the screen (arrow). Click on the little blue icon indicated by the top left arrow.

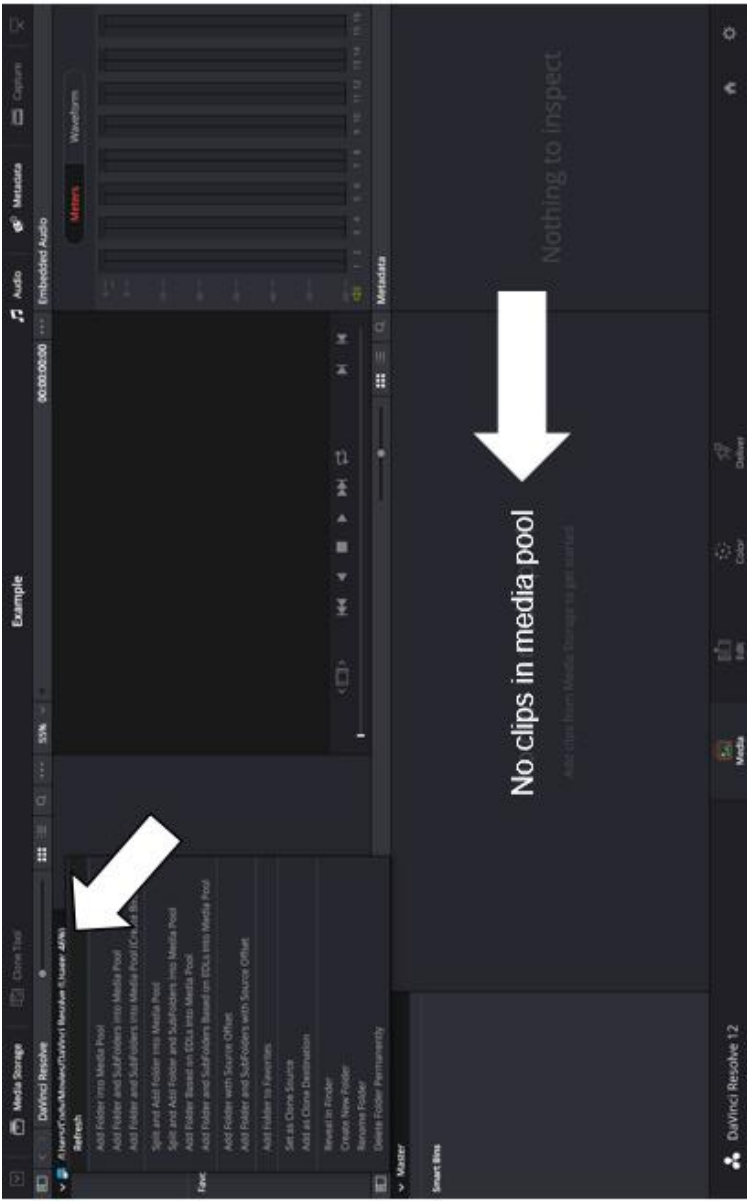


Figure 19. After the time-stamped videos have been placed in the correct directory, return to the *Davinci Resolve* time-stamping functions by right-clicking the directory shown in the top left and clicking refresh (upper arrow). Lower arrow points to "No clips in media pool" (c.f. Figure 20).

Figure 20. At this point the video(s) should be listed in the box directly next to the top left box (top arrow). The videos should be selected and dragged down into the center box that is labeled "No clips in media pool" (see Figure 20). A box may pop up explaining that the framerate specified in the project settings is different from the video files. If this occurs, select "Change" and continue. The videos dragged down should be listed in the bottom middle box (middle arrow). Continue by pressing the "Edit" button (bottom arrow).

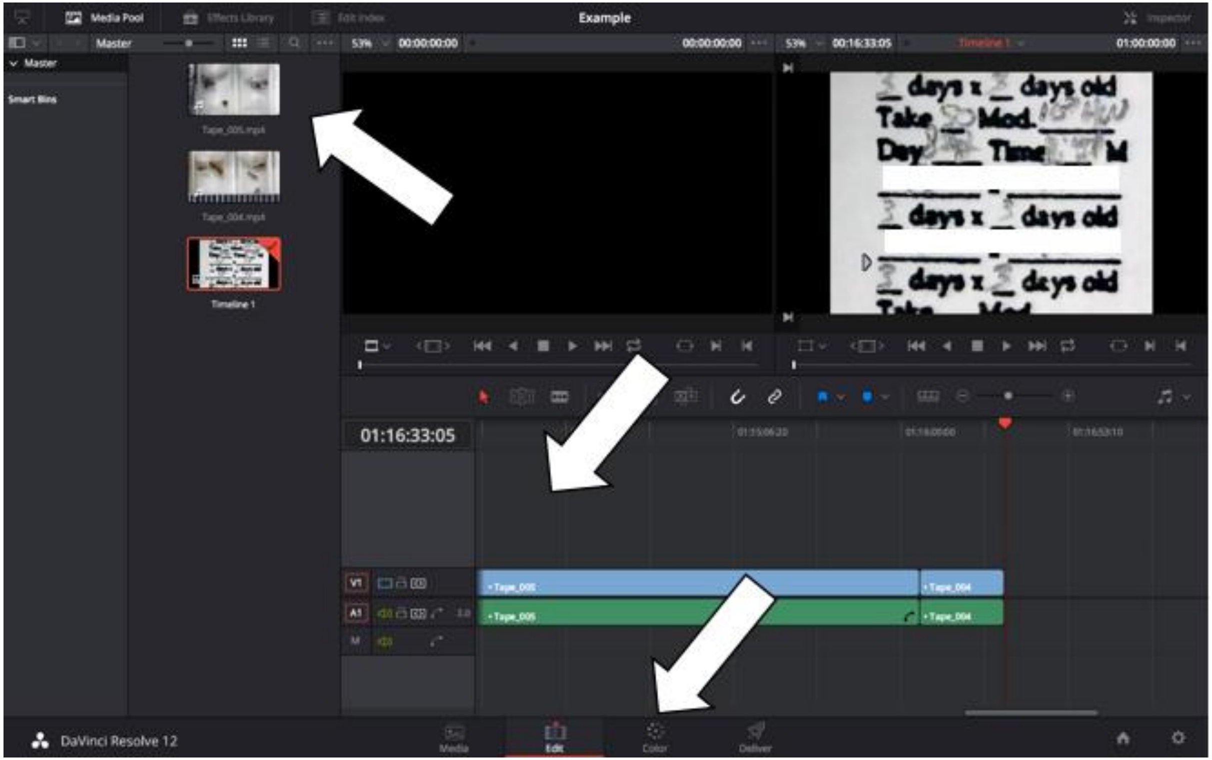


Figure 21. On the edit page the user will be presented with a window listing the video(s) put in the media pool (top arrow) and showing a timeline (middle arrow). Select all the video(s) shown in the left-hand column (top arrow), click on the desired video), and drag it (them) to the timeline box (middle arrow) until the blue and green horizontal bars that appear while dragging the video(s) are flush with the left-hand side of the timeline box before releasing it(them). The window should look similar to the figure above. After the video(s) are properly placed in the timeline continue by clicking the "Color" button (bottom arrow).

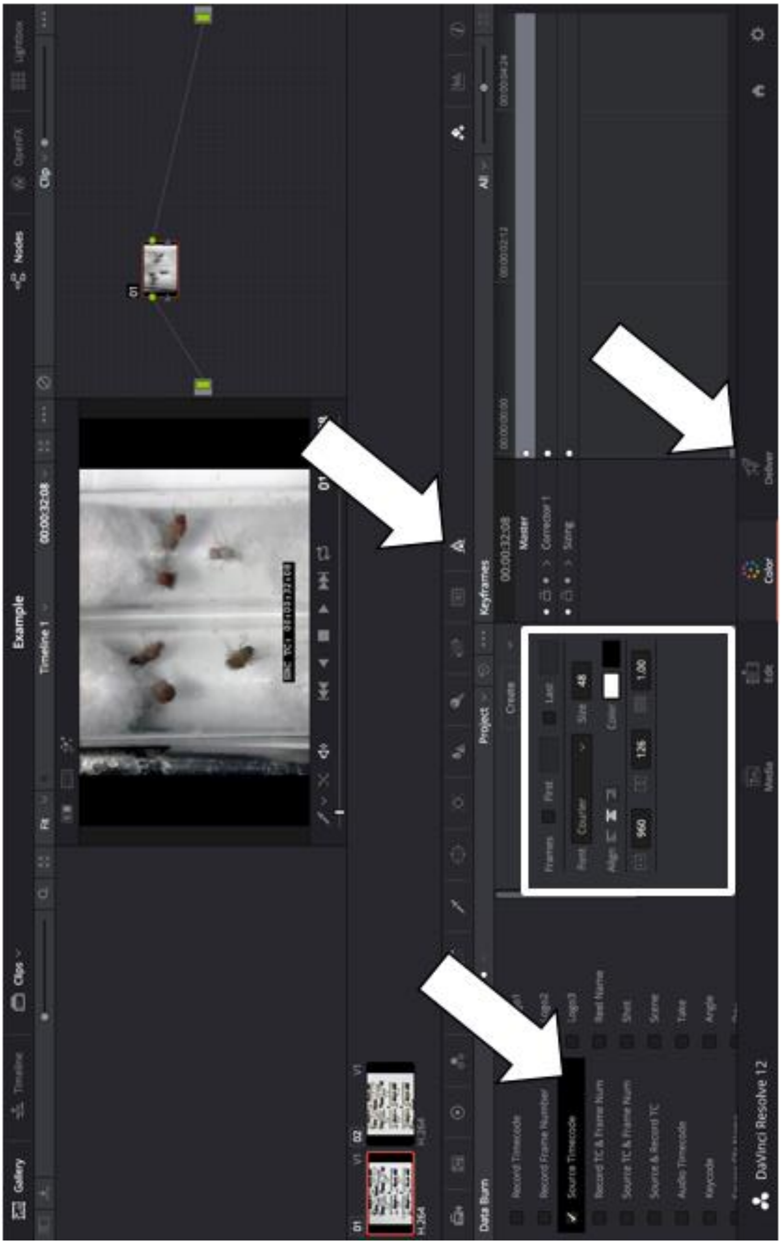


Figure 22. On the Color Page, the user will be presented with a row of buttons across the center of the window. Select "Data Burn" (top arrow). After selecting "Data Burn", new options will be shown below. Check the "Source Timecode" box (left arrow). After selecting "Source Timecode", options (boxed) to change the font, size, positioning, etc. of the timecode will be shown. After the user is satisfied with the settings continue by clicking the "Deliver" button (bottom arrow).

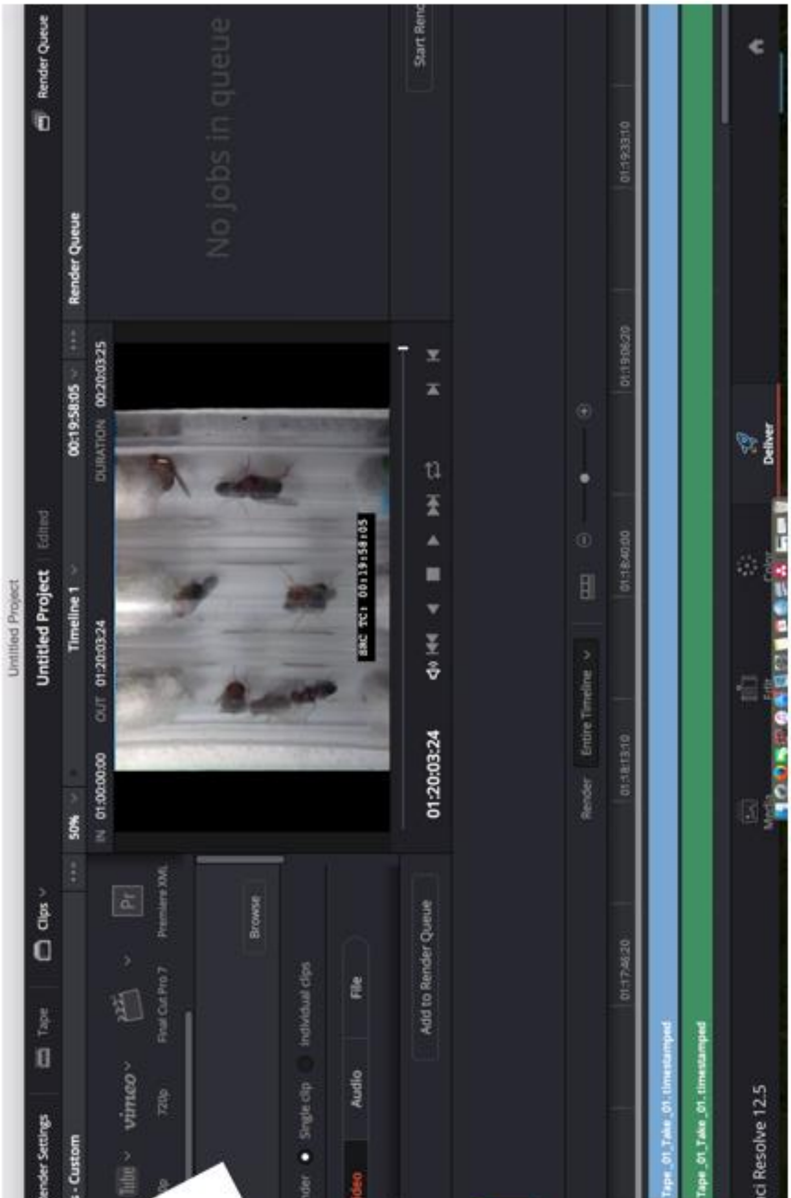


Figure 23. Click on the check mark located on the upper left hand corner (arrow) to expand the side screen (Figure 24).

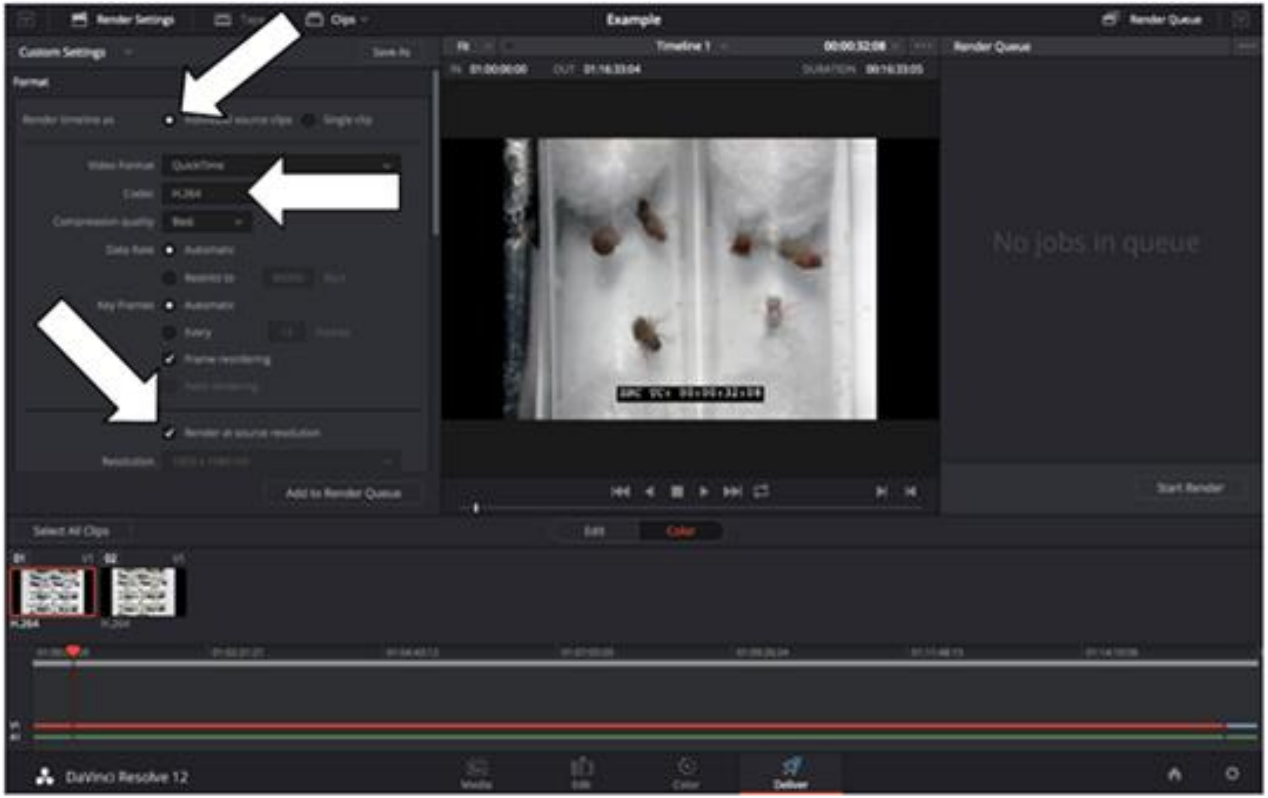


Figure 24. On the Deliver Page, the user is presented with final rendering options along the left. Make sure the settings match what is shown in the figure above. The "individual source clips" selection (top arrow) will keep the videos from rendering as one long video if the user is rendering multiple video files. The Codec selection (middle arrow) of H.264 denotes the method used to encode the video. The H.264 codec keeps the file size manageable without greatly reducing the file quality. Selecting the "Render at source resolution" checkbox (bottom arrow) ensures that the video is rendered at its original resolution. Additional options are shown on Figure 25.

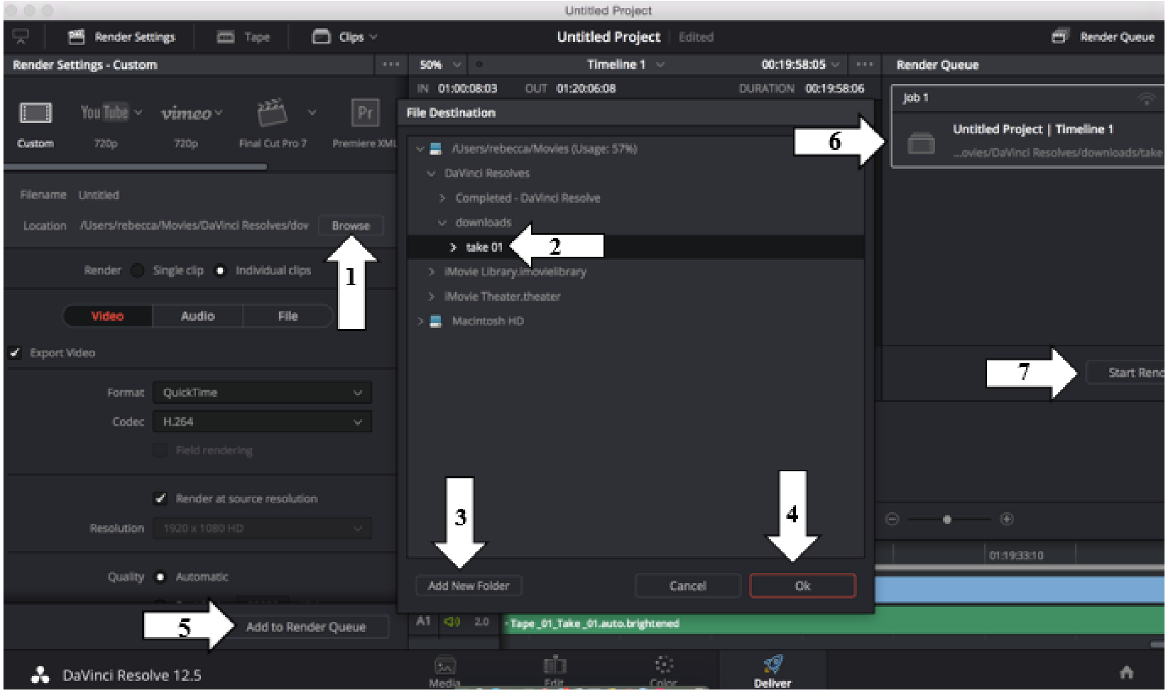


Figure 25. This figure is similar to the previous but shows other options that are presented in the left-hand column, most importantly, the file saving settings. To save the file, click on “Browse” (arrow 1) located on the leftmost column of the screen. This will save the completed files with the same name as the source files. It is a good idea to save the video files to a folder where they are easily found after they have been time-stamped. A box, entitled “File Destination” will appear. Select the location where the file should be placed (arrow 2) and then click "New Folder" (arrow 3). Name this new folder "take 01" as shown and click OK (not shown). This is where the finished video(s) will be placed. The user should see that the newly created folder is now listed in the window beneath the original directory. Make sure that the desired video is selected and click OK (arrow 4). After these selections are finalized, click the "Add to Render Queue" button (arrow 5). A new job will appear in the Render Queue right-hand column (arrow 6). Click the "Start Render" button (arrow 7) to begin the rendering process. This could take a very long time depending on video(s) size and the power of the computer being used (c.f. Figure 26). When rendering is complete, the video(s) will be found in the *DaVinci Resolve* directory within the "Completed" folder created earlier (refer to Figure 19).

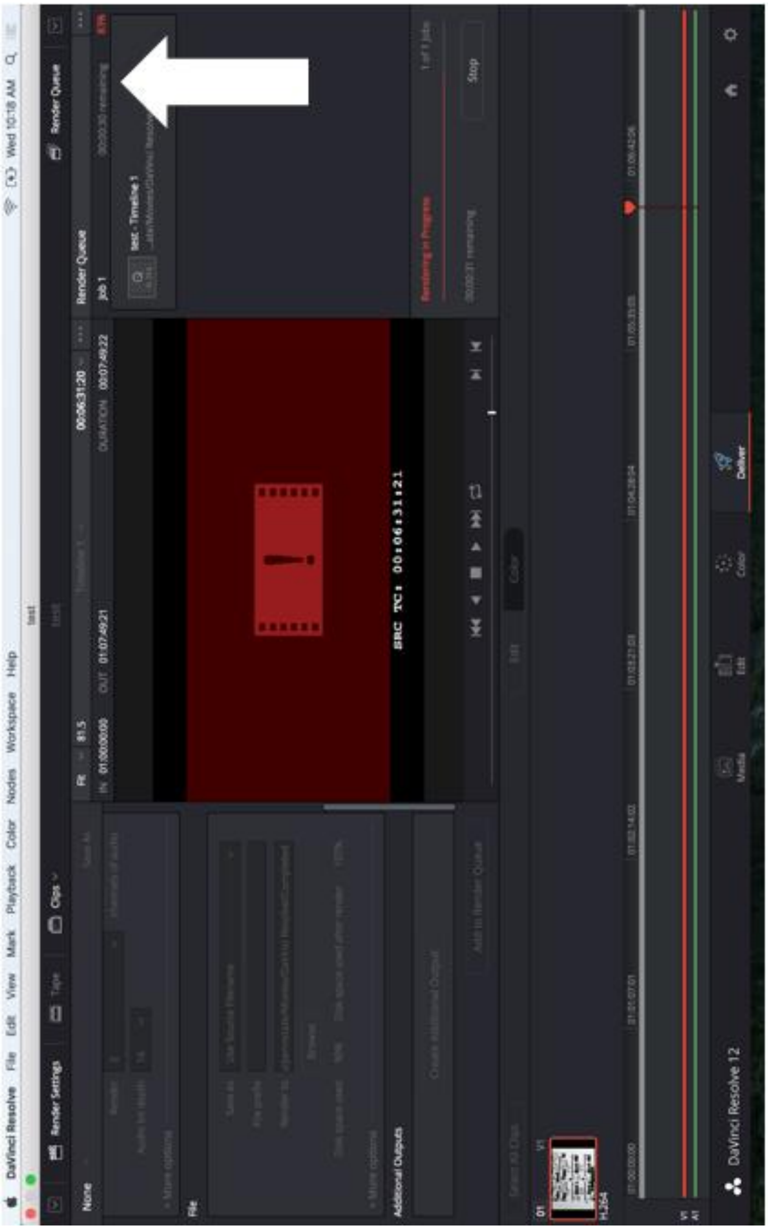
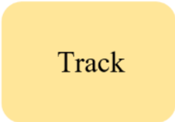


Figure 26. As the rendering is progressing, DaVinci Resolve gives the user a continuous status report (estimated time to completion and percentage of rendition completed.

Tracking the movements of objects using OpenCV



We used the *OpenCV* library (version 2.4.11)[9] to manipulate videos (Bradski 2000). The protocol is illustrated in Figures 27 – 34. This open source library, with bindings for, or the ability to communicate with, programs written in *C++*, *Pytho*n, *Java*, *Matlab*, and others, is a cross-platform library that provides routines for the extraction and manipulation of frames from video. The class files (with the exception of main.cpp, which is the main field) used to perform the tracking functions (analyzeFrame.h , GlobalDefinitions.h , JVCDistortion , LineDesc.cpp , LineDesc.h , main.cpp , PotentialBug.cpp , PotentialBug.h , tracker.cpp , tracker.h , trackerAnalysis.cpp , trackerAnalysis.h , varianceTracker.cpp , and varianceTracker.h) were written by coauthor Caprio using *C++* using the *GCC C++* compiler (version 5.3.1). We have made the codes available, as pdfs of text files, in this site: https://blaypublishers.com/2016/11/22/opencv-files-of-santiago-blay-et-al/. Anyone who wishes to run the program will have to re-enter the entire texts. The compiler then compiles all those files into a new executable file. Although the files available through the link are listed alphabetically, they are called into action as the main tracking program are invoked.

The techniques used are based on routines for video surveillance. The goal of most tracking software is to isolate objects that do not move, or the "background", from objects that are moving, the "foreground". These terms have nothing to do with the relative position of objects and only refer to still or moving objects. Tracking objects in videos normally starts by taking a standard reference frame with no subjects in it as in previous work with bedbugs (*Cimex lectularius* L., Insecta: Hemiptera: Cimicidae) (Goddard et al. 2015). This frame serves as a static background reference model or comparison frame. Each subsequent frame is compared to this initial frame and altered pixel values are assumed to represent moving objects. In the case of these videos, there were no blank frames without insects (Figure 27A). *OpenCV* includes several routines that allow for dynamic background model updating.

Author Caprio developed a hybrid system, using the cv::BackgroundSubtractorMOG2[10] class (Zivkovic 2004) on the initial 1800 frames of each video file to build a statistical model of the reference background. The difference between frame 1800 and the background model constructed from the first1800 frames clearly shows that for the most part the insects have been eliminated from the background model (Figure 27B). While the cv::BackgroundSubtractorMOG2 class can also segment images into background

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[9] For more information on *OpenCV* library, please visit this link: http://docs.opencv.org/trunk/annotated.html#gsc.tab=0 Footnotes 9-15 refer to sites on the huge OpenCV library(OpenCV (Open Source Computer Vision). 2016. http://opencv.org/

[10] http://docs.opencv.org/trunk/d7/d7b/classcv_1_1BackgroundSubtractorMOG2.html#gsc.tab=0

and foreground objects, we found it to be less responsive to rapid movements in the video than attempts with this hybrid method. We called this estimate a static background image and used custom routines to segment the image into foreground anc brackground objects. In normal practice the cv::BackgroundSubtractorMOG2 would also be used to segment the image.

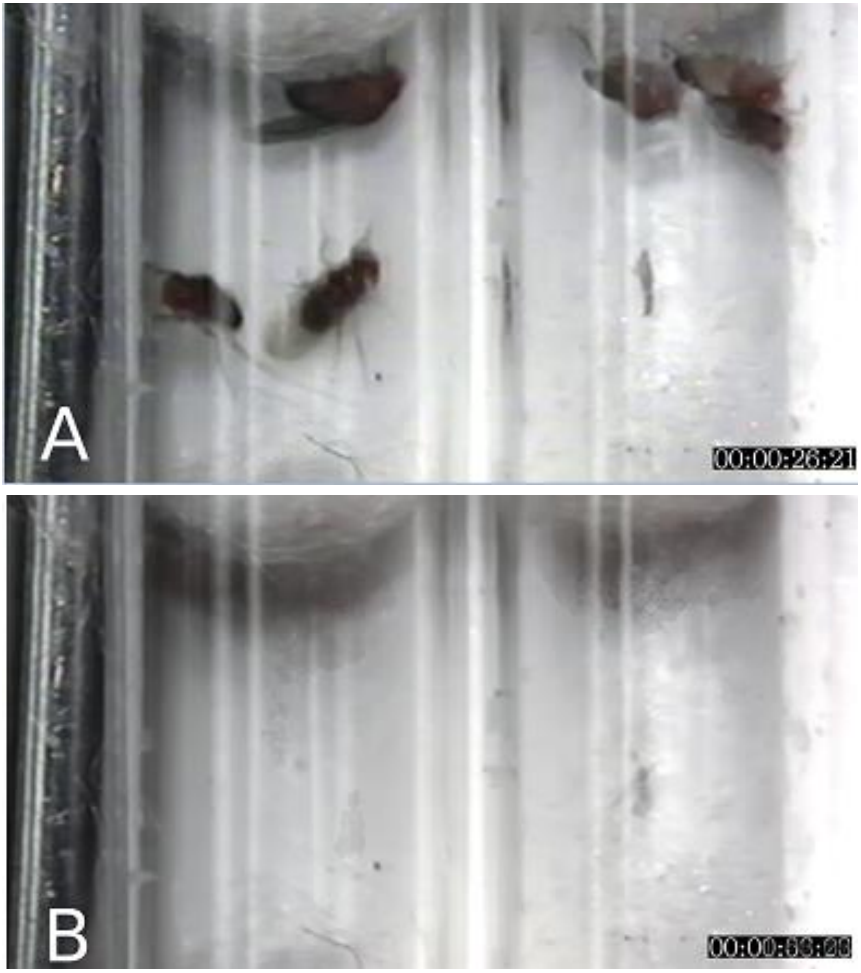

Figure 27. A. The original frame from the video. B. The cv::BackgroundSubtractorMOG2 estimate of the static background reference without insects of the same frame.

Once a suitable reference background model was created by cv::BackgroundSubtractorMOG2 class, the next task was to segment each image into a black and white frame where white pixels corresponded to the foreground and black pixels corresponded to the background. The video was rewound to the initial frame and individual frames loaded one at a time. Each pixel in the current frame was compared to the same pixel in the background reference frame using three filters. These filters were applied simultaneously on copies of the frame, not sequentially. The first filter was a color filter (cv::inRange())[11] that, using high and low values for each color channel (RGB), rejected pixels that fell outside this range (turned them black) while all other pixels were turned white.

The second filter was a black and white threshold filter. Both the background reference model and the current video image were temporarily converted to the grayscale color space using cv:cvtColor[12]. The cv::absdiff[13]_frame function was used to identify pixels in the current frame (Figure 27A) with altered values compared to the background model (Figure 27B). Pixels that had changed from the reference image (the difference exceeded a threshold value) indicated the presence of a foreground object and converted to white, while unchanged pixels were converted to black, leaving a binary black and white image (Figure 28). The third filter was similar to the second but used all three channels in the color frames and a three channel threshold was used (i.e., it worked on the original color image). The three black and white images resulting from these three filters were then logically ANDed, creating an image that only contained white where all three filters suggested there was a foreground object. This black and white image (Figure 28) is the raw input to the next phase of foreground tracking, blob detection.

To reduce the occurrence of small random noise, the cv::erode function was applied to this image two times and then the cv::dilate[14] function was applied three times to recover larger patches of white (altered pixels). This image was then submitted to the cv::SimpleBlobDetector[15] class. This is a large class that identifies the white "blobs" in an image and returns a vector of key points for each blob (hopefully insect). This routine offers additional filtering opportunities and can filter on size, circularity, inertia, convexity and other attributes. The centroid of each blob identified was then mapped on the image and compared to a list of the centroids of all blobs detected in the last five images. These were analyzed to find the nearest neighbor to this blob. If that distance was greater than the parameter, TrackerMinDistance, the blob was assumed to be a new blob (previously undetected insect) and entered into the list of potential insects. If the nearest neighbor is closer than that distance, they were assumed to be the same

[11] http://docs.opencv.org/trunk/d2/de8/group__core__array.html#ga48af0ab51e36436c5d04340e036ce981&gsc.tab=0
[12] http://docs.opencv.org/trunk/d7/d1b/group__imgproc__misc.html#ga397ae87e1288a81d2363b61574eb8cab&gsc.tab=0
[13] http://docs.opencv.org/trunk/dc/da1/structcv_1_1cudev_1_1absdiff__func.html#gsc.tab=0
[14] http://docs.opencv.org/trunk/d4/d86/group__imgproc__filter.html#ga4ff0f3318642c4f469d0e11f242f3b6c&gsc.tab=0
[15] http://docs.opencv.org/trunk/d0/d7a/classcv_1_1SimpleBlobDetector.html#gsc.tab=0

object. A line was drawn from the blob's previously reported position to its new position and its position updated in the list. To increase accuracy of the nearest neighbor routine, all 29.97 frames/sec were analyzed, even if movement data might be required over longer time steps (e.g., object position once a second). Longer time steps, if required, would be calculated by dropping frames after analysis.

Figure 28. A frame of the video showing the items identified as foreground objects. The cv::SimpleBlobDetector was applied to this image to extract the centroid and size of each object.

Once the video clip has been analyzed, the user is presented with the initial reference image overlayed with all the tracks of potential insects identified during analysis (Figure 29). Clicking on any track will cause a text file to be output with the track name. This text file consists of columns of the x and y pixel positions of the centroid of the blob along with the frame reference (the number of frames since the start of the video). Converting pixel positions to physical distance requires some scale to be present in the video. The analysis software may, for various reasons, split the track of a single individual into multiple tracks, but the text versions of the tracks can be easily recombined and otherwise edited. They are easily imported into spreadsheets for further analysis as csv (comma separated values) files. Tracks can also be reviewed frame by frame (with a frame counter onscreen) to assist in correlating behaviors with specific frames.

The output is in the form of two files. First, a *video* that represents the path of the flies in the take (Figures 29-31). In these files, each fly path is represented by a different color, further enhancing the user's ability to recognize the fly.

Second, an *editable* spreadsheet (Figure 32) of *x* and *y* coordinates, as well a time coordinate (in frames). Any errors that the software made (e.g. occlusions of the visual overlap of the path of two or more objects) during the motion tracking are fixed. Spreadsheets allow users to manually correct such errors, restoring the accuracy of the data. Having the data in spreadsheet format allows users to create coded comment columns representing different behaviors facilitating rapid quantification of the observations linked with spatiotemporal information (Figures 33-34).

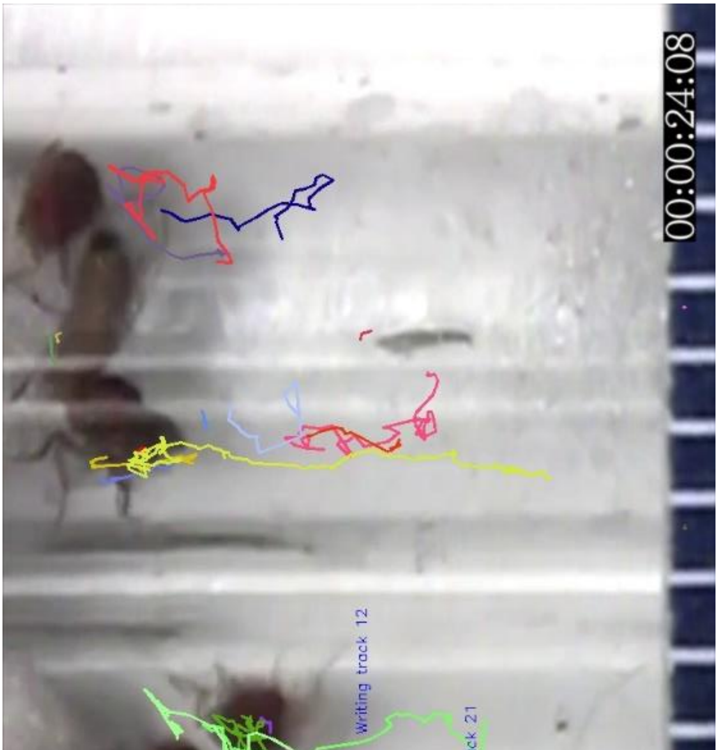

Figure 30. The raw tracks from the analysis of a subset of 129 frames of video. Tracks 12 and 21 (left vial) were combined for subsequent analysis.

Figure 31. The combined track isolated from Figure 29 showing the movement of a single insect over 4.3s. The behaviors can be associated with frame subsets (e.g. from frames 589 to 645 the fly was engaged in a particular activity).

X Coordinate	Y Coordinate	Frame	deltaF	Mean Distance/frame	Mean distance (in pixels)
434.07	222.85	576			
425.52	228.55	577	1	10.27582114	
444.54	225.48	578	1	19.26616983	
435.96	223.94	579	1	8.717109613	
447.06	232.19	580	1	13.83013015	
439.01	232.43	581	1	8.053576845	
443.64	245.03	582	1	13.42374389	
458.38	254.18	583	1	17.34906626	
491.93	253.15	584	1	33.56580701	
490.21	235.85	585	1	17.38529263	
500.71	243.66	586	1	13.08610332	
490.31	239.88	587	1	11.06564051	
496.15	246.82	588	1	9.070237042	
490.68	248.97	589	1	5.877363354	
515.06	242.87	590	1	25.13154193	
517.8	232.43	591	1	10.79357216	78.79
530.25	228.95	592	1	12.92721548	
536.99	223.9	593	1	8.42200095	
545.79	219.37	594	1	9.897519891	
542.62	210.72	595	1	9.212567503	
550.08	202.35	596	1	11.2119802	
549.17	205.24	597	1	3.029884486	
545.78	221.89	598	1	16.99160381	
493.89	224.87	599	1	51.97549904	
524.84	233.1	600	1	32.02554293	
514.68	278.82	601	1	46.83528584	
480.87	271.99	602	1	34.4929703	
485.85	283.71	603	1	12.73415879	
524.92	317.83	604	1	51.87137264	
528.37	337.59	605	1	20.05891572	
530.56	357.7	606	1	20.22889517	64.37323201
533.04	366.04	607	1	8.700919492	
535.16	371.74	608	1	6.081480083	
543	386.25	609	1	16.49259531	
548.63	392.04	610	1	8.075951956	
554.82	387.39	611	1	7.742002325	
561.25	397.62	612	1	12.08295494	
559.14	401.44	613	1	4.364000458	
560.03	410.48	614	1	9.083705191	
554.74	422.99	615	1	13.58249609	
551.67	425.57	616	1	4.010149623	
559.64	435.3	617	1	12.57751168	
558.64	450.77	618	1	15.50228693	
552.71	451.98	619	1	6.052189686	
554.67	447.31	620	1	5.064632267	
549.23	450.44	621	1	6.276185147	63.29731906
541.32	454.44	622	1	8.863864846	
535.57	464.12	623	1	11.25899196	
526.73	471.72	624	1	11.65785572	
518.38	475.81	625	1	9.297881479	
514.62	481.71	626	1	6.996256142	
553.51	487.16	627	1	39.27002165	
446.53	465.03	628	1	109.2449418	arrows
406.01	492.65	629	1	49.0380954	

Figure 32. Raw output (in part) and analysis from the track of Figure 29. The raw data (columns 1-3) can be effortlessly imported into an Excel spreadsheet (this figure) by opening a new Excel file, going to the **File** tab, choosing the **From Text** option, and browsing and choosing the desired file containing the raw data. Columns 4-6 are calculated. The user knows the frame number and thus the time, when did the events occurred. As an example, data denoted by "arrows"(next to last row) is pointed to by arrows in the panels of Figures 33-34.

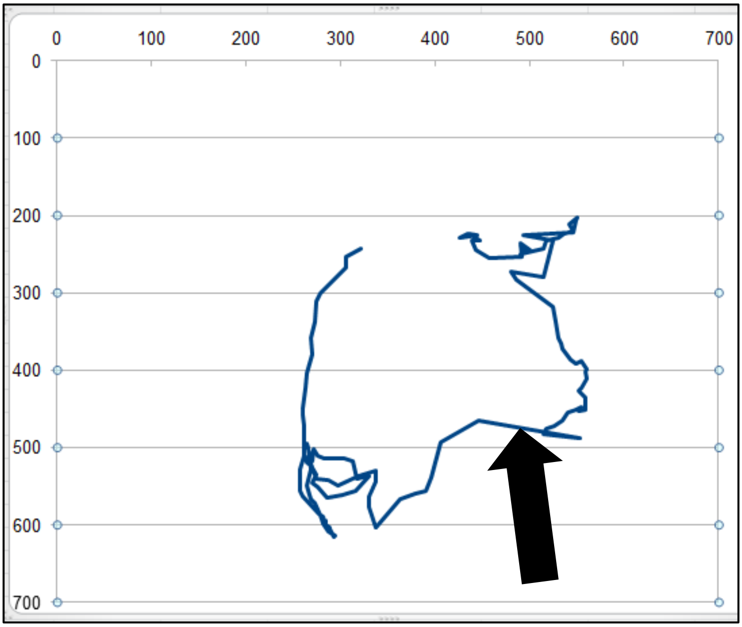

Figure 33. An X-Y plot created in a spreadsheet of the track shown in Figure 30. This represents an alternative method to present and annotate the data. The arrow denotes a sudden burst of movement.

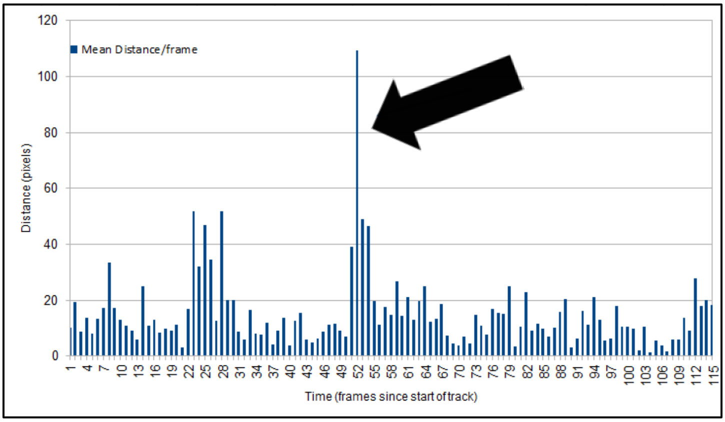

Figure 34. The distance moved by the tracked fly in Figures 29-30. The Euclidian distance was calculated in the spreadsheet from the coordinate data reported by the tracking software. The arrow indicates a sudden burst movement at approximately frame 52 in a background of oscillatingly slow and fast, movements.

Discussion

What can this technology add to the body of the scientific knowledge? We have shown that tracking technology could also be applied to VHS videos taken at the microscopy level to address detailed questions in evolutionary biology, such as the origin and maintenance of species in a more quantitative fashion. A mechanism of speciation whereby gene frequencies in a lineage may change through time and space is behavioral, also known as ethological, through non-random mating. In this phenomenon, also known as sexual selection, individuals of one gender, usually the females, choose a mate based on the traits she can detect on her suitor (Anderson 1994, Gould and Gould 1989, Price 1996, Zimmer and Emlen 2016). In peacocks, females tend to choose males with more "eyespots" in the tail (https://en.wikipedia.org/wiki/Sexual_selection#/media/File:Sexual_Selection_with_Peafowl.gif). By carefully dissecting the behavior of the *Drosophila* flies with the workflow we have shown in this paper, we hope to detect the elements of those behaviors that make males flies more (or less) successful with their conspecific females.

Although nobody knows how many hours of VHS tapes with potentially valuable information there are out there, two anonymous reviewers of an earlier version of this paper said "there must be huge amounts of videotaped research material sitting on shelves gathering dust" and "I am certain great many individuals will be interested in the technical aspects of the work". (A vast amount of data is now potentially easily analyzable by unleashing the awesome power that digital technology provides. Although the tracking software is not perfect – errors caused by objects – the flies - moving on top of or close to others, known as occlusions, need to be corrected manually, the tracking software does the tedious tracking job more quickly and accurately than a human can and frees the investigator to do what s/he is best at, annotating the spreadsheet with the observed behaviors and interpreting them.

In summary, the workflow (Figure 1) herein presented is, as follows. First, transform analog data into digital (Figures 3-11), potentially unleashing the awesome power of digital technology. Second, improve the visual appeal of the newly digitized video (Figures 12-15). This is also important as tracking software operating on low-quality digitized video will yield nearly useless digital videos. Third, time-stamp the digitized video such that it is visible on the computer screen as the user will need to study, correct, and interpret the video on the spreadsheet (Figures 16-25). Fourth, track the movement data, correcting it as needed[16], using computer vision technology, as shown in this paper (Figures 26-33).

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[16] The tracking software yields x, y and time coordinates automatically but, as always, the human user needs to check the output as the tracking is not perfect.

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Erratum

Jorge A. Santiago-Blay

The back cover of ***Life: The Excitement of Biology*** 4(2) had a typo. The paper written by Puente et al. begins on page 88, not page 87 as mistakenly stated by me.