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1 Title: BatCount: A software program to count moving animals

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- 16 species lack reliable population information to guide management decisions. Current approaches
- 17 for estimating population sizes of bats in densely occupied colonies are time-intensive, may
- 18 negatively impact the population due to disturbance, and/or have low accuracy. Research-based
- 19 video tracking options are rarely used by conservation or management agencies for animal
- 20 counting due to the perceived training required to operate. In this paper, we present BatCount, a
- 21 free software program created in direct consultation with end-users designed to automatically
- 22 count aggregations of bats at cave roosts with a streamlined and user-friendly interface. We
- 23 report on the software package and provide performance metrics for different recording habitat

conditions. Our analysis demonstrates that BatCount is an efficient and reliable option for
counting bats in flight and has important implications for range- and species-wide population
monitoring. Furthermore, this software can be extended to count any organisms moving across a
camera including birds, mammals, fish or insects.

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29 Introduction:

30 Effective species management and conservation hinges on accurate population 31 information. For species that are cryptic and/or difficult to count, such as bats, traditional 32 population estimates including visual, photographic counts, or mark/recapture techniques are prone to bias (1). Furthermore, many methods to estimate populations require observers to enter 33 34 caves or roosts, disturbing threatened and endangered species during sensitive time periods that 35 may cause bats to abandon their roost, such as during the maternity season when adults care for 36 young. Additionally, entering caves can result in potentially exposing a colony to the pathogenic 37 fungus responsible for white-nose syndrome (2,3). Due to these limitations, populations of most 38 major bat caves are monitored less than would be desired to establish presence/absence at roosts, 39 calculate population trends over time, or gain additional life history information on the timing 40 and duration of seasonal migrations. As a result, we lack fundamental information on the 41 population of many bat species worldwide, especially species that are currently listed as 42 threatened or endangered. This lack of reliable population information for bats remains a priority 43 for many agencies including the U.S. Fish and Wildlife Service (4). 44 In the past several years, advances in technology have made thermal video systems more

user friendly and affordable, and many researchers and governmental agencies now use thesecameras to record animals in the darkness. Over a decade ago, the U.S. Army Corps of Engineers

created proprietary software ("T³") integrated into a camera system to count bats from thermal 47 48 imagery (5), but the software has not been maintained and cannot be used with current thermal imaging cameras. Recent advances in machine learning approaches and image analysis toolboxes 49 50 have resulted in several algorithms for tracking the movements of animals (6–11), yet these 51 products have not been widely used by users outside of academia, largely due to the perceived 52 training required to run the software (M. Armstrong, personal communication; V. Kuczynska, 53 personal communication; N. Sharp, personal communication). As a result, the few thermal 54 imagery population estimations conducted by biologists outside of academic institutions are 55 achieved with manual counts of video samples, which is a time-intensive process. 56 Motivated by the desire for a free, user friendly counting program that requires little 57 training and can be integrated with video formats from different camera manufacturers and 58 models, we developed BatCount software. This software was designed in collaboration with U.S. 59 Fish and Wildlife Service biologists, with a goal of quick adoption among management and 60 conservation agencies. Due to its intuitive graphical user interface, this software does not require 61 the user to have expertise in any coding languages, and as such is appropriate for broad use 62 among researchers, students, and even the general public. Furthermore, by paring down the 63 output results of the software and including a summary table and output video, we have 64 simplified the results to include the information most relevant to end users. Although created 65 with the main application of counting bats, due to the modifiable input parameters this software 66 can also be used to count birds, mammals, fish or insects.

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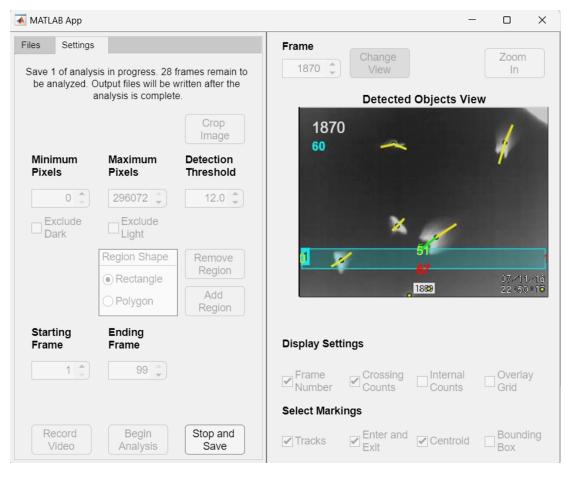
70 Materials and Methods

71 Availability and hardware requirements

72	BatCount v1.24 was developed using MATLAB R2022a (MATHWORKS, Natick, MA)
73	and runs on Windows (Mac OS version in testing). The software uses a standalone interface that
74	does not require the user to purchase or install MATLAB. Rather, specific MATLAB routines
75	and toolboxes that are needed are automatically installed during the software installation.
76	Minimum hardware requirements to operate the software include 4 GB RAM and 2 GB video
77	card RAM, with 24 GB RAM and 4 GB video card RAM recommended for optimal
78	performance. Testing of the software was conducted with three different thermal cameras: 1) A
79	Viento 320 (Sierra-Olympic, Hood River, Oregon) with 320 x 240 resolution recording at 30
80	frames per second, 2) A FLIR Scion OTM 266 (Teledyne FLIR, Wilsonville, Oregon) with 640 x
81	480 resolution recording at 30 frames per second, and 3) a FLIR Photon (FLIR, Wilsonville,
82	Oregon) with 320 x 240 resolution recording at 30 frames per second. The software install file,
83	source code, and user guide can be downloaded at
84	http://sites.saintmarys.edu/~ibentley/imageanalysis/pages/BatCount.html.
85	
86	BatCount algorithm
87	BatCount v1.24 first allows users to upload a video for analysis from its graphical user
88	interface. The program supports videos in multiple formats including .avi, .gif, .mj2, .mov, .mpg,
89	.mp4, and .wmv at any resolution and any frame rate. The program uploads videos and partitions

- 90 the videos into smaller video segments to improve performance as the video is analyzed. Its
- 91 interface then allows users to a preview any frame of the selected video, navigate between
- 92 frames, and edit the image for the preview (e.g., crop, zoom). The user can specify the frame

93 range in which to count bats, the maximum and minimum pixel range in which to consider an 94 object a bat, and the threshold, which determines the detection level in which the software will 95 detect an object against the background. The user can also specify one or multiple regions of interest for tracking, which can be either a rectangle or a polygon with user specified vertices. 96 97 Additionally, users can choose to ignore all objects that are either lighter or darker than the 98 background. The final user-specified inputs include preview display settings (frame number, 99 crossing counts, internal counts, and overlay grid) and output video settings (tracks, enter and 100 exit, centroid, and bounding box). An example of the software interface is depicted in Figure 1.





102 Figure 1: BatCount user interface with a video loaded and the detected object's view

toggled on. This image was taken during an analysis of a video, so many of the adjustable user
 parameters appear grayed out and not editable at this stage. A rectangular region of interest has

105 been specified on this frame to count the number of bats that pass through it. The bats' overall 106 flight trajectory starts from the top of the image and continues toward the bottom portion of the screen, intersecting the rectangular region along their path. The frame number 1870 is shown in 107 108 white, and the crossing sum (60 in this case), which calculates the bats that move through the 109 rectangular region, is displayed below it. A net count of 51 bats have entered the top of the 110 region (shown in green), 67 have left the bottom of the region (shown in red), one net bat has 111 exited the right (shown in red) and no net bats have exited the left side (note the blue highlighted 112 1, which indicates the number of the selection box, slightly obscures the yellow 0 below). See S1 113 Video for the original video file used for this analysis, Table S1 for corresponding summary 114 output table and S2 Video for the software output video file. Note: for ease of visibility in the 115 manuscript we electronically manipulated the contrast of the box counting numbers due to partial 116 occlusion by the box and tracking line.

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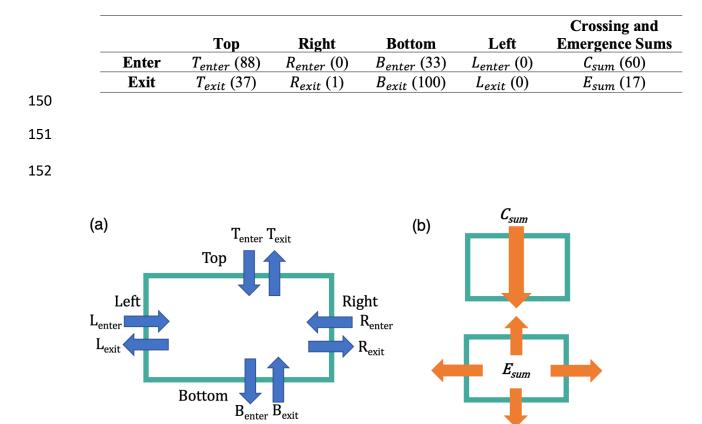
118 The software operates by detecting moving foreground objects (bats) against a 119 background. To account for motion relative to a static background, we use an adaptive process 120 for background determination by calculating the median value of the local segment of video 121 frames (as discussed in (12)). We also re-calculate the relationship between the background and 122 exiting bats over the video duration because the background color will continually change as a 123 result of dropping temperatures and resulting heat loss from the background surface at sunset. 124 The local segmented video frames are used so that the overall lighting is comparable between the 125 background and the frame of interest. The use of a median value as a background is based on the 126 reasoning that if bats are present at any given pixel for fewer than half of the frames, then the 127 median value will contain only background.

128 The tracking phase of the software results in a count of bats moving across the user 129 specified regions. The software determines connecting lines ("tracks") relating the center of one 130 detected object across subsequent frames using a nearest neighbor approach. More specifically, 131 the tracks are calculated by comparing three sequential frames. First, the center of a bat is 132 determined in the current frame and the prior frame. Based on these positions the center is 133 predicted for where a bat should be on the future frame, assuming linear motion. If the predicted 134 location is within the bounding box for a bat in the future frame, then a line is drawn indicating a 135 correctly predicted future track. The same process is run backward to determine prior tracks. The 136 corresponding tracks for forward and backward tracks are used to determine if a bat has entered 137 or exited a user specified region of interest. These crossing counts are ultimately used to 138 determine overall counts for the videos.

Upon completion of the counting, the software outputs 4 files: 1) an output summary table, 2) an output settings file, 3) a detailed counting log of the number of bats both in the entire frame and in the region of interest, and 4) if specified by the user, an output video overlaid with detected objects and tracks. An example of an output summary table is shown in Table 1 with the corresponding explanation of the output results table illustrated in Figure 2. See supplemental information for example test video (S1 Video) and corresponding output files (S1 Table and S2 Video).

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147 Table 1: Example output summary table. See Eq. 1 and 2 for explanation of the crossing and148 emergence sums. The values represented in parentheses are the actual values calculated for the



149 example shown in Figure 1 (see also S1 Video).



Figure 2: Illustration of the output summary results table based on the user selected region. This example shows the output for a rectangular box. (a) The software counts the total number of bats entering and exiting each side of the selection box for the entire video. (b) illustration of the bat flight profiles that would be appropriate for using the Crossing sum, C_{sum} (Eq. 1), and Emergence sum, E_{sum} (Eq. 2). The C_{sum} should be used when counting bats transiting across the user selected region, whereas the E_{sum} should be used when counting bats emerging from a central position within selected region.

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163 The software compiles the enter and exit values as bats cross each region of the rectangular box 164 or polygon, as well as calculates two summation metrics. The crossing summation metric, C_{sum} , 165 sums the number of bats if bats are moving across the field of view of the camera in one 166 generally polarized direction, such as bats emerging from a cave opening. For a rectangular 167 region of interest this is calculated by summing the larger of the entering or exiting values on 168 each side:

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$$C_{sum} = \frac{1}{2}(|(T_{>} - T_{<}) + (B_{>} - B_{<}) + (L_{>} - L_{<}) + (R_{>} - R_{<})|)$$
 Eq. 1

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where T denotes the top side, B denotes the bottom, L denotes the left, and R denotes the right
(Figure 2). Here the greater than and less than correspond to the greater or and lesser, values of
the entering count and the exiting counts. This automatic determination of the largest value,
between enter and exit counts, allows for counting of bats crossing the camera's field of view in
any direction. In the crossing sum, the values are divided by 2 to account for the double
counting of the same bat entering a region of interest on one side and exiting on another, such as
a bat moving from left to right or top to bottom.

The emergence summation metric, E_{sum} , corresponds to the number of bats leaving or entering a region of interest, such as if bats are emerging from a bat box, tree, pit cave, or if the camera was pointed directly facing a cave opening. This is calculated by:

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183
$$E_{sum} = |(T_{enter} - T_{exit}) + (B_{enter} - B_{exit}) + (L_{enter} - L_{exit}) + (R_{enter} - R_{exit})|$$
Eq. 2
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185 where the difference in respective number of bats entering and exiting each side is calculated.

For videos where there is bulk movement across the region of interest the C_{sum} metric is 186 greater and for videos where there is bulk movement into or out of a region of interest the E_{sum} 187 188 metric is larger. Both output counts are saved in the output data file and the larger of the two 189 values is displayed in the interface below the frame number. For example, Table 1 depicts the 190 actual counts in the summary output file for the example illustrated in Figure 1. The value listed at the top of the selection box in Figure 1 $T_{enter} - T_{exit} = 51$, corresponds to 51 more bats 191 entering (green) the top than had exited. Similarly, the net value $R_{enter} - R_{exit} = -1$, 192 193 corresponds to one more bat exiting (red) the right then had entered. Similarly, $L_{enter} - L_{exit} =$ 0 corresponds to no net bats traveling across the left portion, and $B_{enter} - B_{exit} = -67$, 194 195 corresponds to 67 more bats exiting the bottom than entering. Based on these count differences: $C_{sum} = 59.5$, which has been rounded to 60 and is displayed below the frame number and 196 written in cyan to match the cyan color region of interest. $E_{sum} = 17$, and while displayed in the 197 198 summary output table is not visible on the software interface because it is the smaller of the two numbers. If E_{sum} was greater than C_{sum} , its value instead would be displayed and shown in cyan. 199 200 See S1 Video for the original video file used for this analysis, Table S1 for corresponding 201 summary output table and S2 Video for the software output video file.

It is important to emphasize that the user should think carefully about the count values most appropriate for their video. For example, C_{sum} was designed for videos in which bats are truly crossing opposing regions of the selection box, i.e., top to bottom or left to right. For some recording scenarios, bats may be entering crossing adjacent corners, such as entering from the top and exiting the right. In these situations, relying on C_{sum} will substantially undercount the bats, and it would instead be better to use the enter and exit counts from one region of the selection box, such as the top. As such, users of the software should always preview the

emergence video to determine the summary table output value that is most appropriate given theoverall bat flight behavior.

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212 *Software accuracy*

213 We evaluated the accuracy of the software with thermal recordings from 8 different 214 locations: 6 Myotis grisecens (MYGR, gray bats) and 2 Tadarida brasiliensis (TABR, Brazilian 215 free-tailed bat) maternity roosts. Date, location, software accuracy, and camera information for 216 each recording is listed in Table 2. We chose videos with different roost types, species, 217 background clutter, bat densities, and emergence profiles to represent the diversity of 218 applications by the end user. Due to the length of recordings and density of bats in the videos at 219 the maternity caves, manual counts of the entire video were prohibitive. Instead, we randomly 220 selected *n* replicates (see Table 1) of 900-frame video segments from each emergence recording 221 for manual counting. Counting was conducted by trained technicians unaware of software 222 program results. During the initial training period, the technicians both unknowingly counted the 223 same video segments and had manual counts within 96.5% of each other. After the training 224 period, technicians unknowingly overlapped 10% of their video segments so we could ensure 225 continued accuracy in counting. Manual counts were conducted with a frame-by-frame analysis 226 using the KMPlayer software (version 4.2.2.58) in 50 frame segments. To expedite counting, we 227 manually counted bats entering and exiting one of the four rectangular regions (the same region 228 and side for each video) and compared the performance of the software to the manual counts.

- Table 2: Recording date (month/day/year), location (county, state), camera type, average number
- of bats per 900-frame segment, number of video segments included in the analysis, and overall

CaveID	Recording date	Location	Camera	Bats/seg	# video segments	Software accuracy
MYGR1	07/17/2020	Camden, MO	Viento	122	20	91.3%
MYGR2	06/25/2021	Taney, MO	FLIR scion	188	20	90.1%
MYGR3	06/25/1021	Wright, MO	FLIR scion	163	20	94.8%
MYGR4	06/22/2021	Oregon, MO	FLIR scion	252	11	72.0%
MYGR5	??/??/2012	Wilson, TN	FLIR photon	146	20	50.8%
MYGR6	08/13/2021	Nelson, KY	FLIR photon	24	20	56.7%
TABR1	06/13/2016	Woods, OK	Viento	771	20	83.6%
TABR2	06/15/2016	Woodward, OK	Viento	718	20	70.8%

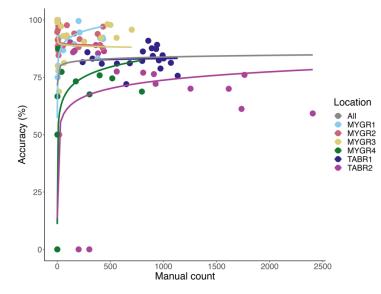
software accuracy for each of the 8 recordings used to evaluate the software.

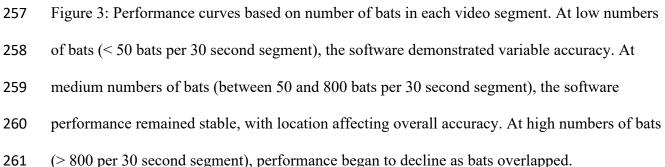
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234 Results and Discussion

235 At the maternity roosts, BatCount software accuracy ranged from 94.8 to 50.8% (Table 236 2). Software performance strongly depended on video quality, with the highest performance 237 achieved for videos with strong contrast between the bats and the background and minimal 238 overlap of bats. Our peak accuracy of 94.8% is slightly higher than the reported accuracy of 93% 239 for the T3 system (5). Camera model also affected performance, with videos recorded by the 240 FLIR Scion and Viento cameras (average performance 85.6 and 81.9%, respectively) 241 outperforming those recorded by the FLIR photon camera (average performance 53.8%). The 242 poor accuracy of the videos MYGR5 and MYGR6 was due primarily to a combination of low 243 background contrast and poor video resolution; even our trained technicians struggled to visually 244 discriminate bats against the background. Therefore, we cannot disambiguate whether the poor

245	performance for these two locations is due to camera quality, environmental conditions, or both.
246	Due to these limitations, we removed MYGR5 and MYGR6 from further analysis.
247	Figure 3 illustrates the accuracy of the software as a function of the number of bats in
248	each 900-frame (30 second) segment for each cave location. At all locations, the software
249	underestimated bat counts. The data are best represented overall with a logarithmic fit, in which
250	accuracy is low at low numbers of bats but remains relatively stable for medium densities of
251	bats. When bats began to overlap at higher emergence densities (TABR1, TABR2), the chance of
252	the software counting two bats as one increased, and accuracy begins to decline. We are
253	currently developing a neural network approach to better count overlapping bats and expect an
254	increase in software accuracy with its incorporation. All updates of the software will be released
255	on the software website and announced via authors' social media.





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263 Although the exact processing time of the software depends on the length of the video 264 and number of bats in each recording, we can make some general statements about the software 265 processing time. Using the minimum hardware requirements listed to run the software, the 266 software processes approximately 1 frame per second. Computers with the recommended 267 specifications can process approximately 2 frames per second. For example, an emergence that 268 lasts 60 minutes and was recorded at 30 frames per second would take approximately 15 hours to 269 process. This time can be partitioned by counting specific segments of the longer emergence 270 video. We also found it helpful to run the counting software overnight. In comparison, our 271 trained technicians manually counted the more challenging videos at a rate of 1 frame every 2 272 minutes. Thus, with standard PC equipment our software can count bats 250 times faster than 273 human effort and reduces human bias. The speed of the software can be further accelerated by 274 using a supercomputer, which should be able to process an entire emergence video in less than a 275 second. The next step for this software is integration into the ground-truthing component of a 276 method to estimate animal populations with passive acoustics (13).

277 In conclusion, with our performance testing we know that the current version of our 278 software is highly accurate when recording gray bats with a high-resolution camera. Future 279 releases of the software will increase performance for dense bat flights. By developing the 280 software in close consultation with and testing from end-users, we have developed a counting 281 software that is intuitive, easy to use, and provides informative summary output including total 282 counts and an output video. This software eliminates the need to exhaust our most precious 283 resource as a conservation community-time. We are currently working with end-users to 284 develop and implement best practices for both placement of cameras in the field and placement

- of the user-defined selection boxes for software counting. This software provides a free and
- powerful tool to obtain population counts of bats emerging from roosts and can be a valuable
- 287 resource to aid in population estimation and species conservation.
- 288

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- 300
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