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Supplemental information

LabGym: Quantification of user-defined animal

behaviors using learning-based holistic assessment

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Figure S1. The stable-value detection method outperforms the state of the art, related to Figure 2.

A. Illustrations showing the designing rationale of stable-value detection method for reconstructing the static background of a video in two different scenarios (animal lighter or darker than the backgrounds).

B. Examples for reconstructed static backgrounds of the same videos using different methods. Arrows

point at the remaining animal traces in the reconstructed static backgrounds.

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Figure S2. The design of the Categorizer, related to Figure 3.

A. The Animation Analyzer first uses time-distributed convolutional layers to compute all frame-wise
 spatial details of an animation, and then uses recurrent layers (long short-term memory, LSTM) to
 compute the temporal connectivity among the frame-wise spatial details.

- 7 B. The Pattern Recognizer uses convolutional layers to analyze the pattern image, which is
- 8 superimposed contours of animal body (and body parts) along the temporal sequence (indicated by
 9 gradually changing colors) during a behavior.
- **C**. The Decision Maker uses a concatenating layer to integrate the outputs from both the Animation
- 11 Analyzer and the Pattern Recognizer, and then passes the integrated information to dense layers for
- 12 concluding the behavioral categories.



Figure S3. LabGym generates stand-alone, visualizable behavior examples, related to Figure 4.

A. Every frame of an animation (background is included) and its paired pattern image, showing nest building behavior of a mouse.

6 B. Every frame of animations and their paired pattern images (body parts are not included), showing

(from top to bottom) larva curling and uncoiling, fly abdomen bending (copulation attempt) and wingextension (courtship song), and rat walking and rearing.

9 **C**. Every frame of animations and their paired pattern images (body parts are included), showing (from

10 top to bottom) mouse facial grooming, sniffing, ear grooming, and sitting.



Figure S4. The Quantifier analyzes diverse aspects of a behavior, related to Figures 5 and 6.

A schematic that shows the process of frame-wise categorizations on behaviors, and how the Quantifier uses the information of behavioral categories and animal foregrounds to calculate 14 behavioral parameters in *LabGym*.

LabGym version 1.5	• • •	Generate Behavior Examples
	Select the video(s) to generate behavior examples	Can select multiple videos.
Welcome to LabGym!	Select a folder to store the generated behavior examples	Will create a subfolder for each video under this folder.
version 1.5	Specify when generating behavior examples should begin (unit: second)	Default: at the 1st second.
Developed by Yujia Hu	Specify how long generating examples should last (unit: second)	Default: lasts until the end of a video.
Bing Ye Lab, Life Sciences Institute University of Michigan	Specify the number of animals in a video	Default: 1.
	Specify the scenario that fits your experiments best	Background subtraction is used to detect animals in each frame.
Generate Behavior Examples	Specify the time window for background extraction	A time window during which the animals are NOT static.
Train Categorizers	Specify the time window for estimating the animal size	A time window during which all the animals are present in the video.
Test Categorizers	Specify the number of frames for an animation	The duration of a behavior episode (should be the same across all behaviors).
Analyze Behaviors	Specify how many frames to skip when generating two consecutive behavior examples	Default: no frame to skip (generate a behavior example every frame).
		Start to generate behavior examples



- Figure S5. The benchmark comparison between DLC + B-SOiD and LabGym, related to Figure 7.
- A. The GUI in LabGym demonstrated with Generate Behavior Examples functional unit.
- 5 **B**. The evaluation of training in DLC on the training dataset.
- 6 7 C. The best fit of clustering (5 groups) in B-SOiD using the outputs from DLC on the training dataset.
- D. The behavior assignment of the 5 groups clustered by B-SOiD on the training dataset. All video
- 8 examples in group 2 showed head movement behavior and group 2 was assigned as 'head movement':
- 9 most of the videos examples in group 0 and group 3 showed rearing behavior and group 0 and group 3 10
- were assigned as 'rearing'; most of the video examples in group 4 showed locomotion behavior and
- 11 group 4 was assigned as 'locomotion'; none of the video examples in all groups showed complete 12
- orientating behavior; most of the video examples in group 1 showed in place resting behavior and group 1 13 was assigned as 'in place'.

LarvaN (c	verall accura	cy: 0.98)		
Animation Analyzer 8 x 8 x 1, level 1				
Pattern Recognizer	32 x 32 x 3, level 2			
1312	precision	recall	f1	
crawling	0.99	0.97	0.98	
curling	0.97	0.97	0.97	
immobile	0.98	1	0.99	
rolling	0.97	0.98	0.97	
turning	0.98	0.98	0.98	
uncoiling	0.96	0.95	0.96	
Categorizer for Lar	vae #2 (over	all accurac	y: 0.93)	
Animation Analyzer	8 x	8 x 1, leve	1	
Pattern Recognizer	32 x	32 x 3, lev	el 3	
	precision	recall	f1	
crawling	0.94	0.96	0.95	
curling	0.92	0.88	0.9	
immobile	0.97	0.95	0.96	
rolling	0.94	0.95	0.95	
turning	0.89	0.93	0.91	
uncoiling	0.92	0.91	0.91	

Catagoria das D			0.74)		
Categorizer for Ra	ats #1 (overa	I accuracy	r: 0.74)		
Animation Analyzer	16 X 16 X 1, level 2				
Pattern Recognizer	32 X	32 x 3, Iev	er s		
	precision	recall	11		
body grooming	0.69	0.73	0.71		
face grooming	0.48	0.68	0.56		
head swaying	0.66	0.74	0.7		
locomotion	0.91	0.91	0.91		
orientating	0.8	0.8	0.8		
rearing	0.8	0.65	0.72		
in place	0.58	0.56	0.57		
still	0.89	0.82	0.85		
Categorizer for B	ats #2 (overa	all accurac	v: 0.8)		
Animation Analyzer	32 x	32 x 1. lev	el 4		
Pattern Recognizer	64 v	64 x 3, lev	el 4		
r attern necognizer	nrecision	recall	f1		
body grooming	0.78	0.71	0.74		
face grooming	0.78	0.71	0.74		
head swaving	0.05	0.5	0.57		
locomotion	0.74	0.70	0.75		
orientating	0.95	0.95	0.95		
rearing	0.88	0.91	0.89		
rearing	0.8	0.85	0.83		
in place	0.69	0.65	0.67		
still	0.8	0.86	0.83		
Categorizer for B	ats #3 (overa	llaccuracy	. 0 76)		
Animation Analyzer	32 x 32 x 1. level 5				
Pattern Recognizer	64 x 64 x 3 level 5				
rattern needgnizer	nrecision	recall	f1		
hody grooming	0.8	0.6	0.69		
face grooming	0.53	0.56	0.54		
head swaving	0.33	0.50	0.76		
locomotion	0.72	0.75	0.70		
orientating	0.05	0.00	0.00		
roaring	0.81	0.05	0.82		
in along	0.78	0.61	0.79		
in place	0.62	0.61	0.61		
sun	0.84	0.84	0.84		
RatA (overall accura	cv: 0.88) (aft	er refining	laheling)		
Animation Analyzor	27 v	22 x 22 x 1 lovel 4			
Pattorn Recognizer	52 x 52 x 1, level 4				
rattern Recognizer	04 X	04 X 04 X 3, IEVEI 4			
hadu groom in -	precision	recall	11		
body grooming	0.89	0.88	0.88		
face grooming	0.82	0.8	0.81		
head swaying	0.75	0.88	0.81		
locomotion	0.95	0.98	0.96		
orientating	0.96	0.92	0.94		
rearing	0.88	0.88	0.88		
in place	0.87	0.7	0.77		
still	0.89	0.93	0.91		

Categorizer for Mi	ce #1 (overa	Il accuracy	y: 0.85)	
Animation Analyzer	32 x 32 x 1, level 2			
Pattern Recognizer	32 x 32 x 3, level 2			
	precision	recall	f1	
behind the wheel	0.94	0.94	0.94	
body grooming fv	0.67	0.44	0.53	
body grooming sv	0.82	0.62	0.71	
chewing fv	0.63	0.68	0.65	
chewing sv	0.74	0.85	0.79	
coming down	0.89	0.99	0.94	
face grooming fv	0.66	0.83	0.74	
face grooming sv	0.86	0.67	0.75	
foraging fv	0.85	0.89	0.87	
foraging sv	0.82	0.93	0.87	
rearing up	0.84	0.83	0.83	
resting on the wheel	0.91	0.92	0.92	
running on the wheel	0.93	0.93	0.93	
sniffing fv	0.59	0.57	0.58	
sniffing sv	0.87	0.74	0.8	
standing	0.93	0.87	0.9	
turning front	0.71	0.75	0.73	
turning side	0.78	0.67	0.72	
unknown bv	0.94	0.9	0.91	
walking	0.93	0.9	0.91	
MouseH (d	overall accur	acy: 0.9)		
MouseH (Animation Analyzer	overall accur 64 x	acy: 0.9) 64 x 1, lev	vel 4	
MouseH (Animation Analyzer Pattern Recognizer	overall accur 64 x 64 x	acy: 0.9) 64 x 1, lev 64 x 3, lev	vel 4 vel 4	
MouseH (d Animation Analyzer Pattern Recognizer	overall accur 64 x 64 x precision	acy: 0.9) 64 x 1, lev 64 x 3, lev recall	vel 4 vel 4 f1	
MouseH (d Animation Analyzer Pattern Recognizer behind the wheel	overall accur 64 x 64 x precision 0.94	acy: 0.9) 64 x 1, lev 64 x 3, lev recall 0.91	vel 4 vel 4 f1 0.93	
MouseH (a Animation Analyzer Pattern Recognizer behind the wheel body grooming fv	overall accur 64 x 64 x precision 0.94 0.71	acy: 0.9) 64 x 1, lev 64 x 3, lev recall 0.91 0.83	vel 4 vel 4 f1 0.93 0.77	
MouseH (a Animation Analyzer Pattern Recognizer behind the wheel body grooming fv body grooming sv	overall accur 64 x 64 x precision 0.94 0.71 0.85	acy: 0.9) 64 x 1, lev 64 x 3, lev recall 0.91 0.83 0.79	vel 4 f1 0.93 0.77 0.82	
MouseH (d Animation Analyzer Pattern Recognizer behind the wheel body grooming fv body grooming sv chewing fv	overall accur 64 x 64 x precision 0.94 0.71 0.85 0.75	acy: 0.9) 64 x 1, lev 64 x 3, lev recall 0.91 0.83 0.79 0.72	rel 4 rel 4 f1 0.93 0.77 0.82 0.73	
MouseH (c Animation Analyzer Pattern Recognizer behind the wheel body grooming fv body grooming sv chewing fv chewing sv	overall accur 64 x 64 x precision 0.94 0.71 0.85 0.75 0.89	acy: 0.9) 64 x 1, lev 64 x 3, lev recall 0.91 0.83 0.79 0.72 0.78	rel 4 f1 0.93 0.77 0.82 0.73 0.83	
MouseH (a Animation Analyzer Pattern Recognizer behind the wheel body grooming fv body grooming sv chewing fv chewing sv coming down	overall accur 64 x 64 x precision 0.94 0.71 0.85 0.75 0.89 0.96	acy: 0.9) 64 x 1, lev 64 x 3, lev recall 0.91 0.83 0.79 0.72 0.78 0.96	rel 4 f1 0.93 0.77 0.82 0.73 0.83 0.96	
MouseH (d Animation Analyzer Pattern Recognizer behind the wheel body grooming fv body grooming sv chewing fv chewing sv coming down face grooming fv	overall accur 64 x 64 x precision 0.94 0.71 0.85 0.75 0.89 0.96 0.85	acy: 0.9) 64 x 1, lev 64 x 3, lev recall 0.91 0.83 0.79 0.72 0.78 0.96 0.93	rel 4 f1 0.93 0.77 0.82 0.73 0.83 0.96 0.89	
MouseH (Animation Analyzer Pattern Recognizer behind the wheel body grooming fv body grooming sv chewing fv chewing sv coming down face grooming fv face grooming sv	overall accur 64 x 64 x precision 0.94 0.71 0.85 0.75 0.89 0.96 0.85 0.83	acy: 0.9) 64 x 1, lev 64 x 3, lev recall 0.91 0.83 0.79 0.72 0.78 0.96 0.93 0.83	rel 4 f1 0.93 0.77 0.82 0.73 0.83 0.96 0.89 0.83	
MouseH (c Animation Analyzer Pattern Recognizer behind the wheel body grooming fv body grooming fv chewing fv chewing fv chewing fv coming down face grooming fv face grooming sv foraging fv	overall accur 64 x 64 x precision 0.94 0.71 0.85 0.75 0.89 0.96 0.85 0.83 0.93	acy: 0.9) 64 x 1, lev 64 x 3, lev recall 0.91 0.83 0.79 0.72 0.78 0.96 0.93 0.83 0.92	rel 4 f1 0.93 0.77 0.82 0.73 0.83 0.96 0.89 0.83 0.93	
MouseH (d Animation Analyzer Pattern Recognizer behind the wheel body grooming fv body grooming fv chewing fv chewing sv coming down face grooming fv face grooming sv foraging fv foraging sv	overall accur 64 x 64 x precision 0.94 0.71 0.85 0.75 0.89 0.96 0.85 0.83 0.93 0.85	acy: 0.9) 64 x 1, lev 64 x 3, lev recall 0.91 0.83 0.79 0.72 0.78 0.96 0.93 0.83 0.92 0.94	rel 4 f1 0.93 0.77 0.82 0.73 0.83 0.96 0.89 0.83 0.93 0.9	
MouseH (d Animation Analyzer Pattern Recognizer behind the wheel body grooming fv body grooming sv chewing sv coming down face grooming fv face grooming sv foraging fv foraging sv rearing up	overall accur 64 x 64 x precision 0.94 0.71 0.85 0.75 0.89 0.96 0.85 0.83 0.93 0.85 0.92	acy: 0.9) 64 x 1, lev 64 x 3, lev recall 0.91 0.72 0.78 0.96 0.93 0.83 0.93 0.93 0.92 0.94 0.92	rel 4 f1 0.93 0.77 0.82 0.73 0.83 0.96 0.89 0.83 0.93 0.93 0.92	
MouseH (d Animation Analyzer Pattern Recognizer behind the wheel body grooming fv body grooming sv chewing fv chewing sv coming down face grooming fv face grooming fv foraging fv foraging sv rearing up resting on the wheel	overall accur 64 x 64 x precision 0.94 0.71 0.85 0.75 0.89 0.96 0.85 0.83 0.93 0.85 0.92 0.97	acy: 0.9) 64 x 1, lex 64 x 3, lex recall 0.91 0.83 0.79 0.72 0.78 0.96 0.93 0.83 0.92 0.94 0.92 0.85	rel 4 f1 0.93 0.77 0.82 0.73 0.83 0.96 0.89 0.83 0.93 0.93 0.92 0.9	
MouseH (d Animation Analyzer Pattern Recognizer behind the wheel body grooming fv body grooming sv chewing fv chewing sv coming down face grooming fv foraging fv foraging sv rearing up resting on the wheel running on the wheel	overall accur 64 x 64 x precision 0.94 0.71 0.85 0.75 0.89 0.96 0.85 0.83 0.93 0.85 0.93 0.85 0.92 0.97 0.91	acy: 0.9) 64 x 1, lev 64 x 3, lev recall 0.91 0.83 0.79 0.72 0.78 0.96 0.93 0.83 0.92 0.94 0.92 0.85 0.98	rel 4 f1 0.93 0.77 0.82 0.73 0.83 0.96 0.89 0.83 0.93 0.92 0.92 0.9 0.94	
MouseH (c Animation Analyzer Pattern Recognizer behind the wheel body grooming fv body grooming fv chewing fv chewing sv coming down face grooming fv face grooming sv foraging fv foraging fv foraging sv rearing up resting on the wheel running on the wheel sniffing fv	overall accur 64 x 64 x precision 0.94 0.71 0.85 0.75 0.89 0.96 0.85 0.83 0.93 0.83 0.93 0.85 0.93 0.92 0.97 0.91 0.68	acy: 0.9) 64 x 1, lev 64 x 3, lev recall 0.91 0.72 0.78 0.96 0.93 0.83 0.92 0.94 0.92 0.85 0.98 0.98 0.57	rel 4 f1 0.93 0.77 0.82 0.73 0.83 0.96 0.89 0.83 0.93 0.93 0.92 0.9 0.94 0.62	
MouseH (c Animation Analyzer Pattern Recognizer behind the wheel body grooming fv body grooming fv chewing fv chewing sv coming down face grooming fv face grooming fv foraging fv foraging sv rearing up resting on the wheel running on the wheel sniffing fv sv	overall accur 64 x 64 x precision 0.94 0.71 0.85 0.75 0.89 0.96 0.85 0.83 0.93 0.85 0.93 0.85 0.92 0.97 0.91 0.68 0.84	acy: 0.9) 64 x 1, lev 64 x 3, lev recall 0.91 0.72 0.78 0.96 0.93 0.83 0.92 0.94 0.92 0.85 0.98 0.57 0.89	rel 4 f1 0.93 0.77 0.82 0.73 0.83 0.96 0.83 0.96 0.83 0.93 0.92 0.92 0.94 0.92 0.94 0.92 0.94 0.92 0.94 0.94 0.95	
MouseH (a Animation Analyzer Pattern Recognizer behind the wheel body grooming fv body grooming sv chewing fv chewing sv coming down face grooming sv foraging fv foraging sv rearing up resting on the wheel running on the wheel sniffing sv standing	overall accur 64 x 64 x precision 0.94 0.71 0.85 0.85 0.89 0.96 0.85 0.83 0.93 0.85 0.92 0.97 0.91 0.68 0.84 0.94	acy: 0.9) 64 x 1, lev recall 0.91 0.83 0.79 0.72 0.78 0.96 0.93 0.83 0.92 0.83 0.92 0.85 0.98 0.57 0.88 0.57 0.89 0.95	rel 4 f1 0.93 0.77 0.82 0.73 0.83 0.96 0.89 0.83 0.99 0.92 0.92 0.94 0.62 0.86 0.94	
MouseH (a Animation Analyzer Pattern Recognizer behind the wheel body grooming fv body grooming sv chewing sv coming down face grooming fv face grooming fv face grooming sv foraging fv foraging sv rearing up resting on the wheel running on the wheel sniffing fv sitifing fv standing turning front	overall accur 64 x 64 x precision 0.94 0.71 0.85 0.75 0.89 0.96 0.85 0.83 0.93 0.93 0.93 0.92 0.97 0.91 0.68 0.84 0.94 0.76	acy: 0.9) 64 x 1, lev 64 x 3, lev recall 0.91 0.83 0.72 0.78 0.96 0.93 0.83 0.92 0.94 0.92 0.85 0.98 0.57 0.89 0.95 0.81	rel 4 f1 0.93 0.77 0.82 0.73 0.83 0.96 0.89 0.83 0.99 0.92 0.9 0.92 0.9 0.94 0.62 0.86 0.89 0.94 0.79	
MouseH (a Animation Analyzer Pattern Recognizer behind the wheel body grooming fv body grooming sv chewing fv chewing sv coming down face grooming fv face grooming fv foraging sv rearing up resting on the wheel running on the wheel sniffing fv sitanding turning front turning front	overall accur 64 x 64 x precision 0.94 0.71 0.85 0.75 0.89 0.96 0.85 0.83 0.93 0.83 0.93 0.85 0.92 0.97 0.91 0.68 0.84 0.94 0.94 0.92	acy: 0.9) 64 x 1, lex 64 x 3, lex recall 0.91 0.72 0.72 0.78 0.96 0.93 0.83 0.92 0.94 0.92 0.94 0.92 0.88 0.57 0.89 0.57 0.89 0.57 0.81 0.79	rel 4 f1 0.93 0.77 0.82 0.73 0.83 0.96 0.89 0.83 0.93 0.93 0.92 0.92 0.94 0.62 0.86 0.86 0.94 0.74 0.74 0.75	
MouseH (c Animation Analyzer Pattern Recognizer behind the wheel body grooming fv body grooming fv chewing fv chewing fv coming down face grooming fv foraging fv foraging fv foraging fv foraging sv rearing up resting on the wheel running on the wheel sniffing fv satifing fv standing turning front turning front turning side unknown bv	overall accur 64 x 64 x precision 0.94 0.71 0.85 0.75 0.89 0.96 0.85 0.83 0.93 0.85 0.93 0.93 0.93 0.97 0.91 0.68 0.84 0.94 0.76 0.92 0.97	acy: 0.9) 64 x 1, lex 64 x 3, lex recall 0.91 0.72 0.78 0.96 0.93 0.83 0.92 0.94 0.92 0.85 0.98 0.57 0.89 0.57 0.89 0.55 0.89 0.57 0.89 0.95 0.79 0.96	rel 4 rel 4 f1 0.93 0.77 0.82 0.73 0.83 0.96 0.89 0.89 0.83 0.93 0.92 0.94 0.62 0.86 0.94 0.79 0.85 0.97	

Table S1. Example showing the training metrics for all tested Categorizers in selecting the onesthat are most suitable for each of the 3 behavioral datasets, related to Figure 4.We started from the simplest networks for each dataset and gradually increase the network complexity

until the training performance was satisfying.