# A distribution-dependent analysis of open-field test movies

## Tomokazu Konishi, Akita Prefectural University

This is a protocol of open field test for checking activity of animals. Movement of the animals are recorded by a small digital camera, digitized by the Image (<https://imagej.nih.gov/nih-image/>), and analyzed parametrically on the R (<https://cran.r-project.org/>). Examples and the basis of analysis are published elsewhere.

# Animal test

Plastic container boxes are used as the open field. Those are available at DIY stores. Choice of the colors is important to obtain enough contrast to the animal. A particular part of the animals can be colored and spotted. Otherwise, the shape of the animals should be separated from the box background in black and white images.

# Recording

Almost any small digital camera or movie camera can be used. No extra illuminations will be required. Fix the camera with a tripod and take the movie from straight above.

Start to record the movie before the animals are put in the box. This will give vacant images, which can be used as the background of images to be subtracted; illumination in the box will have certain unevenness, and this has to be compensated by using the background. The minimum resolution would be enough. The format has to be a changeable one to .avi.

# Digitizing using “the Image”

## Input the data

The Image can read movies recorded in.avi format. The Image stores the movie as a stack of many images, so the size of the movie will become very huge: PCs with large size of RAM are recommended.

### Format changes

As many of the digital cameras and movies do not use .avi format, the record format of the movie has to be changed. This could be performed with some freeware, such as XMediaRecord. During this process, the framework of the movie can be cropped. Additionally, the resolution can be reduced. In general, the resolution of digital cameras is too much for the purpose (pixels of ca 150x150 would be enough to cover a 50x50 cm field).

## The Image

### Import the movie to the Image

The resulted .avi file can be imported into the Image (drag and drop will start the importing). The movie can be recorded as gray scale (this will reduce the size of the image; the whole shape can be recognized in BW images). Select to use stacks. 30 min movie will make a stack of 54,000 images.

### Duplicate the stack

Image > duplicate > “duplicate stack”

This allows the Image to alter the movie.

### Optional: invert the brightness

Edit > Invert

This will ease to subtract the background. Black animals on white background should to this process.

### Make an appropriate background

Process > Image Calculator ~~> “duplicate stack”~~

The opened stack, without animals or human hands, will be duplicated.

### Subtract the background from the movie

Process> Image Calculator

“the duplicated movie” “subtract” “the background”

The unevenness of the illumination will be removed by this process.

### Binarization

To define the shape of animals, the gradation tone of each image is simplified into black and white, emphasizing the edge of the animals in the images.

Image > Adjust > Threshold

### Find the position and area of the animal

Analyze > Set measurements. Check “Area” “Center of mass” “Display label”

Analyze > Analyze particles

The “Results” can be saved as a tab-separated text (maybe through Excel).

# Analyze the information

## Input to the “R”

The tab-separated text can be imported to the R. The R-code is in the appendix. The movement is analyzed on several constant distribution patterns, finding out important parameters.

There are several points that requires manual tunings. See the comments in the code.

# Appendeix

sample.avi: a short binarized movie

area.txt: the recorded data output of the Image

code.txt: the R code of analysis