

Jun 23, 2024

## ilastik install and run for Syn Bot (Windows Version)

 In 1 collection

DOI

**[dx.doi.org/10.17504/protocols.io.5jyl82eedl2w/v1](https://dx.doi.org/10.17504/protocols.io.5jyl82eedl2w/v1)**

Justin T Savage<sup>1</sup>

<sup>1</sup>Duke University

ASAP Collaborative Rese...

Eroglu\_Lab



Justin T Savage

Duke University

OPEN  ACCESS



DOI: **[dx.doi.org/10.17504/protocols.io.5jyl82eedl2w/v1](https://dx.doi.org/10.17504/protocols.io.5jyl82eedl2w/v1)**

**Protocol Citation:** Justin T Savage 2024. ilastik install and run for Syn Bot (Windows Version). **protocols.io**  
**<https://dx.doi.org/10.17504/protocols.io.5jyl82eedl2w/v1>**

**License:** This is an open access protocol distributed under the terms of the **[Creative Commons Attribution License](#)**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

**Protocol status:** Working

**Created:** June 20, 2024

**Last Modified:** June 23, 2024

**Protocol Integer ID:** 102176

### Abstract

Video instructions for installing ilastik, training an ilastik project, and using the ilastik project for simple SynBot run for Windows operating system.



1

<https://www.youtube.com/embed/ApOwcOFMUcg?si=azuelTcu87ixq8hy>

- 1.1 If video quality is poor, try watching directly from Youtube at <https://www.youtube.com/watch?v=ApOwcOFMUcg>

## Installing ilastik

- 2 0:00 - 1:30 Install ilastik by downloading it from [ilastik.org/download](https://ilastik.org/download) and running the installer.

## Generate Training Images

- 3 1:30 - 3:00 Generate training images by running SynBot on a set of images so that any preprocessing steps will be the same as a SynBot run.
- 4 3:00 - 4:00 Copy two-color training images to a training images folder
- 5 4:00 - 4:20 Make empty folder for holding red images and green images
- 6 4:20 - 4:32 Run **extract\_channels.ijm** macro from the extra code folder on the SynBot GitHub repository
- 7 4:32 - 5:22 Select merged images folder, then red images folder, then green images folder

## Train ilastik model

- 8 5:22 - 5:39 Open ilastik and start a new Pixel Classification project
- 9 5:39 - 6:01 Save the ilastik project file to a safe place where you can use it to threshold with SynBot



- 10 6:01 - 6:20 Click add new image and select the red training image (or images)
- 11 6:20 - 6:31 Click the "2. Feature Selection" drop down then "select features" and use Ctrl + A to select all features.
- 12 6:31 - 6:34 Click the "3. Training" drop down to advance
- 13 6:34 - 6:41 Zoom in image using Ctrl + +
- 14 6:41 - 7:05 Use the Label 1 to mark several examples of foreground puncta. Only a handful are needed to get started.
- 15 7:05 - 7:21 Click Label 2 and use this to label some examples of background regions.
- 16 7:21 - 7:37 Click Live Update to see a rendering of the ilastik model that will update when new markings are added. Use Ctrl + - to zoom out and check how well this model is working.
- 17 7:37 - 7:49 Save the ilastik project. This saved model will be used by SynBot and you do not need to change anything in the steps 4 or 5 in the ilastik pipeline.
- 18 7:55 - 9:37 Repeat steps 8-17 for the green channel image

## Run SynBot with ilastik thresholding

- 19 9:37 - 9:51 Open and run SynBot.ijm
- 20 9:51 - 10:14 Enter your analysis parameters, including the ilastik thresholding method.
- 21 10:14 - 10:29 Select ilastik.exe application location. On Windows this will typically be in a path like C:/Program Files/ilastik1.4.0/ilastik.exe



- 22 10:29 - 10:34 Select the ilastik project file for thresholding the red channel.
- 23 10:34 - 10:40 Select the ilastik project file for thresholding the green channel.
- 24 10:40 - 10:42 Enter the threshold of ilastik confidence to use for the analysis. ilastik will give a confidence score from 0 to 1 of how likely the given pixel is part of the foreground. A good threshold is something between 0.5 and 0.9.
- 25 10:42 - 12:14 View results