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ilastik install and run for Syn Bot (Mac Version)

In 1 collection

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Abstract

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

1

https://www.youtube.com/embed/NiNJ48GOABo?si=e5669PakvJ4Xy0rS

1.1   If video quality is poor, try watching directly from Youtube at https://www.youtube.com/watch?v=NiNJ48GOABo

Installing ilastik

2   0:00 - 1:00 Install ilastik by downloading it from **ilastik.org/download** and running the installer. To run ilastik after initial installation, right-click the application and click open. You will then be prompted to open the application even though it is from an unrecognized developer.

Generate Training Images

3   1:00 - 2:38 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   2:38 - 3:15 Copy two-color training images to a training images folder

5   3:15 - 3:30 Make empty folder for holding red images and green images

6   3:30 - 3:50 Run **extract_channels.ijm** macro from the extra code folder on the SynBot GitHub repository

7   3:50 - 4:40 Select merged images folder, then red images folder, then green images folder

Train ilastik model

8   4:40 - 4:58 Open ilastik and start a new Pixel Classification project

9   4:58 - 5:09 Save the ilastik project le to a safe place where you can use it to threshold with SynBot

| 10 | 5:09 - 5:25 Click add new image and select the red training image (or images) |

| 11 | 5:25 - 5:35 Click the "2. Feature Selection" drop down then "select features" and use Ctrl + A to select all features. |

| 12 | 5:35 - 5:37 Click the "3. Training" drop down to advance |

| 13 | 5:37 - 5:40 Zoom in image using Cmnd + + |

| 14 | 5:40 - 6:05 Use the Label 1 to mark several examples of foreground puncta. Only a handful are needed to get started. |

| 15 | 6:05 - 6:12 Click Label 2 and use this to label some examples of background regions. |

| 16 | 6:12 - 6:21 Click Live Update to see a rendering of the ilastik model that will update when new markings are added. Use Cmnd + - to zoom out and check how well this model is working. |

| 17 | 6:21 - 6:30 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 | 6:30 - 8:01 Repeat steps 8-17 for the green channel image |

Run SynBot with ilastik Thresholding

| 19 | 8:01 - 8:43 Open and run SynBot.ijm. Move ilastik to your applications folder. FIJI may not have permission to access ilastik otherwise. |

| 20 | 8:43 - 8:59 Enter your analysis parameters, including the ilastik thresholding method. |

| 21 | 8:59 - 9:15 Select ilastik.app application location. On Mac this will typically be in a path like ~/Applications/ilastik-1.4.0.app |

22   9:15 - 9:20 Select the ilastik project file for thresholding the red channel.

23   9:20 - 9:25 Select the ilastik project file for thresholding the green channel.

24   9:25 - 9:30 Enter the threshold of ilastik condence to use for the analysis. ilastik will give a condence 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   9:30 - 10:30 View results