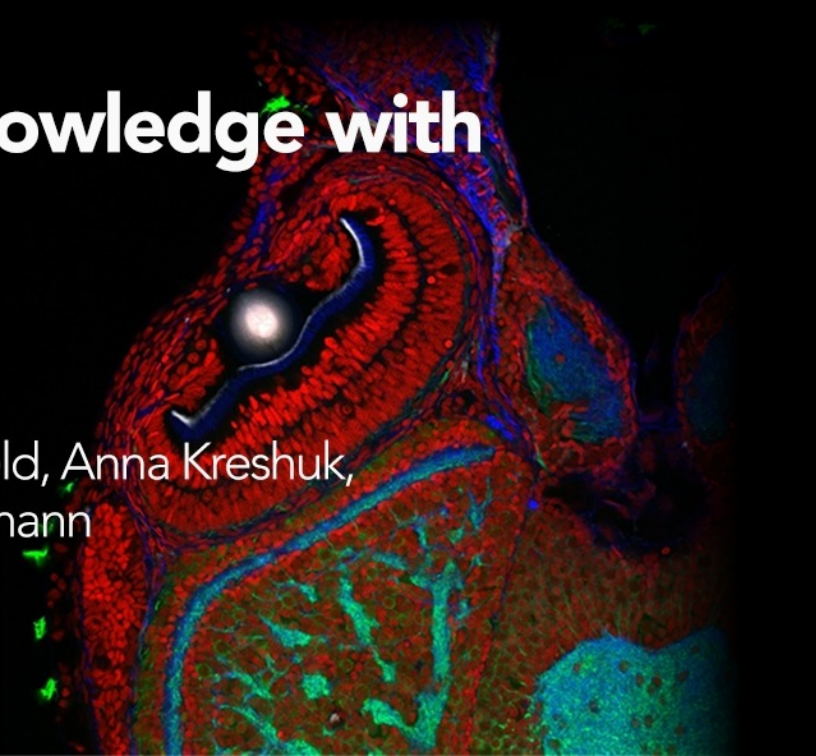# From Images to Knowledge with ImageJ & Friends

virtual conference

Nov 30 - Dec 2, 2020

Stephan Preibisch, Stephan Saalfeld, Anna Kreshuk, Pavel Tomancak and Virginie Uhlmann

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Conferences

Using Annotater to quickly and efficiently annotate or correct nuclei segmentation and identify associated markers in 2D immunofluorescence multiplexed images

Tutors: Thierry Pécot (pecot@musc.edu)

Session 1:   2020-11-30 21:00 UTC – 2020-12-01 01:00 UTC
Session 2:   2020-12-02 13:00 UTC – 2020-12-02 17:00 UTC

Information about the tutor

Dr. Pécot, a CZI Imaging Scientist, is an engineer and applied mathematician with expertise in bioimage informatics. He has developed algorithms and analysis pipelines for a variety of biological applications. Since he joined the Hollings Cancer Center at the Medical University of South Carolina in 2017, he has been developing tools to process immunofluorescence multiplexed images to profile tumor immune status.

Title and abstract of the tutorial

Title: Using *Annotater* to quickly and efficiently annotate or correct nuclei segmentation and identify associated markers in 2D immunofluorescence multiplexed images

Abstract: Coupling immunofluorescence with multiplexed microscopy enables to visualize different cell types in the tumor microenvironment. The quantification of these cells requires to accurately segment nuclei and to identify the associated cell markers. While deep learning has demonstrated astonishing segmentation results for many biological objects acquired with microscopy, the good performance of this technology relies on large training datasets. *Annotater* is an ImageJ plugin that allows to quickly and efficiently annotate objects in microscopic images. It can also be used to correct object segmentation to improve the accuracy or to increase the training dataset used by the deep learning-based segmentation. Additionally, *Annotater* gives the possibility to annotate regions in the image such as epithelium and stroma. From segmented objects, a second functionality of *Annotater* consists in identifying markers associated to segmented objects, either by manually annotating them, by using a thresholding tool or by using a logistic regression. Finally, image features associated with objects and their marker characterization can be summarized in a result file. In addition, a batch process mode is available to identify markers associated with pre-segmented objects and to extract features for a large number of images.

Outline of the tutorial

The goal of the tutorial is to show how to get advantage of *Annotater* to process immunofluorescence multiplexed images, where nuclei were stained with DAPI and 6 other markers were used to characterize immune and epithelial cells. More specifically, the students will learn how to use *Annotater* to annotate/correct nuclei segmentation, annotate the tumor and the stroma and identify the markers associated with the nuclei to characterize the different cell types. The tutorial will be organized as follows:

1. Introduction of the topic (15 min)
2. Introduction of each student, their background and their motivation (2-5min each, 45 min)
3. Practical work and training (2 h 30 min):
    1. Installation and presentation of *Annotater* (visualization panels, analysis panels, annotation panels)
    2. Annotation of nuclei
    3. Correction of nuclei segmentation
    4. Annotation of epithelium and stroma

5. Identification of markers with the thresholding tool
6. Identification of markers with the logistic regression tool
7. Extraction of features (markers or combination of markers associated with nuclei, tissue and nuclei related features) for each nucleus in the image
8. Batch processing (marker identification and feature extraction) of a small number of images
4. Summary and questions (30 min)

Images will be provided. The students will only need a computer with ImageJ running. It is preferable but not required to have a drawing tablet.

Time of the day to host the tutorials

Thierry can host tutorials from 8am to 8pm on Monday, 8am to 5pm on Tuesday and 8am to 3.30pm on Wednesday (US east coast time).

Annotater history

Annotater was first released a year ago. Over the last year, an extensive testing and upgrades have been performed. The plugin was just added to be downloaded via the Fiji update site https://sites.imagej.net/annotater. The plugin code and video tutorials are available at https://github.com/tpecot/Annotater.