XVII INTERNATIONAL SYMPOSIUM ON
SCHISTOSOMIASIS
PERSPECTIVES ON SCHISTOSOMIASIS ELIMINATION
NOVEMBER 10-13TH 2024 | SALVADOR - BAHIA

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TITLE

AI-ASSISTED INSTANCE SEGMENTATION OF SCHISTOSOMA MANSONI SCHISTOSOMULA IN HIGH CONTENT MICROSCOPY IMAGES

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ABSTRACT

Traditionally, the schistosomicidal effect of compounds is evaluated through optical microscopy, monitoring parasite's motility and morphology visually—a process that is slow, subjective, and semi-quantitative. To overcome these limitations, automated assays, such high content microscopy (HCA) have been employed to capture and analyze the images of larval and adult forms of schistosomes. Recently, our group began developing a novel HCA-based assay to screen compounds on Schistosoma mansoni schistosomula. In this study, we show our initial efforts to segment the parasites in the HCA images using an artificial intelligence (AI) model. First, schistosomula were distributed in 96-well microplates and incubated with or without 10 µM praziquantel (PZQ) for up to 72h. Then, timelapse (5 images, 6s interval) brightfield images from four adjacent sites within each well were captured by a ImageXpress Micro Confocal HCA microscope (Molecular Devices) coupled with a 4x objective. Image annotation was carried out with Anylabeling software (Neural Research Lab) using an AI-powered semi-automated data label tool (Segment Anything Model from Meta AI). The JSON files generated in this process, along with their associated images, were employed to fine-tune a mask region-based convolutional neural network (mask R-CNN) model (X101-FPN) available in the detectron2 (Meta AI) package for Python. This step was conducted using a nested cross-validation (CV) technique, with 3-fold and 2-fold splits for the outer and inner CV loops, respectively. In both CV, model performance was evaluated with the mean average precision (mAP) metric considering a 50-95% IoU (intersection over union) threshold interval (mAP50-95). Hyperparameter (number of epochs and learning rate) tunning was carried out with the inner CV training/validation datasets by the optuna package for Python. After 15 optimization trials, the best hyperparameter values were used to train three models, one per outer CV, giving a mean mAP50-95 score of 80.6 ± 1.4 (mean ± standard deviation) for the test datasets. Additionally, the metrics mAP50 and mAP75 were also determined as 96.4 ± 0.4 and 96.3 ± 0.5, respectively. A visual analysis comparing the predicted segmentation masks and those generated by semi-automated annotation corroborated the high mAP scores, as parasites with normal and PZQ-induced phenotypes were consistently identified in the images, even when in contact with each other. In contrast, experimental artifacts, such as the remaining tails of the cercaria, debris and air bubbles were mostly ignored. These results suggest that this segmentation model can be implemented as the first step in the image analysis pipeline, allowing for the subsequent extraction of morphological and motility features from each schistosomula. Our next steps include continuing model optimization with additional hyperparameters, as well as conducting new experiments with parasites exposed to other schistosomicidal compounds.

KEYWORDS

Schistosomula; Instance Segmentation; Artificial Intelligence; High Content Microscopy; Schistosomiasis Drug Discovery.

FINANCIAL SUPPORT

FAPERJ; CNPq; CAPES; FIOCRUZ