FiCRoN, a Fully Convolutional Regression Networks for intracellular amastigote quantification

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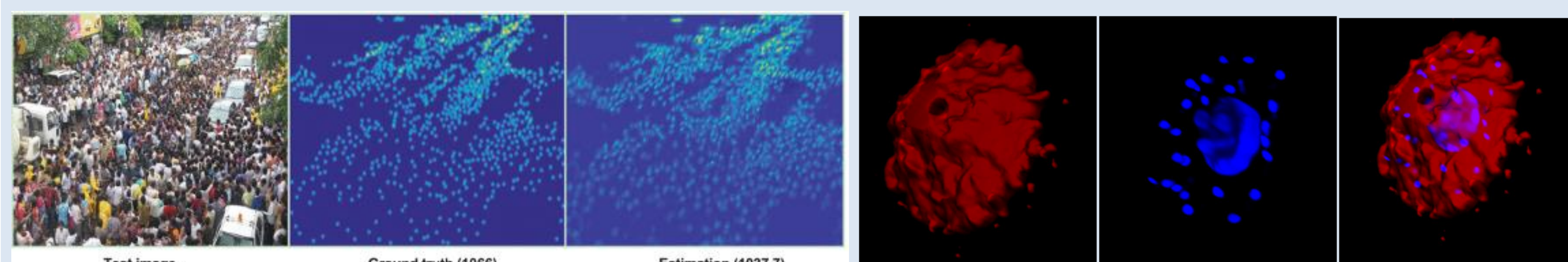
1. Introduction

A fundamental step for the validation of new drug targets and for drug screening requires quantifying intracellular *Leishmania* parasites (amastigotes) within the infected macrophage. This is traditionally done manually by counting stained parasites under light or fluorescence microscopy, using hundreds of images, a very time consuming process. Recently, alternative automatic counting methods have emerged, based on individual cell segmentation and classification processes. However, these conventional image processing methods present difficulties if there is cell overlap, high confluence, or fuzzy borders. These problems can cause overestimation or underestimation of the parasite load. In this work we propose a method based on Fully Convolutional Regression Networks (FiCRoN) as an efficient approach to estimate parasite load from fluorescence microscopy images. In this method, the integral of the density maps is related to the number of cells in the image.

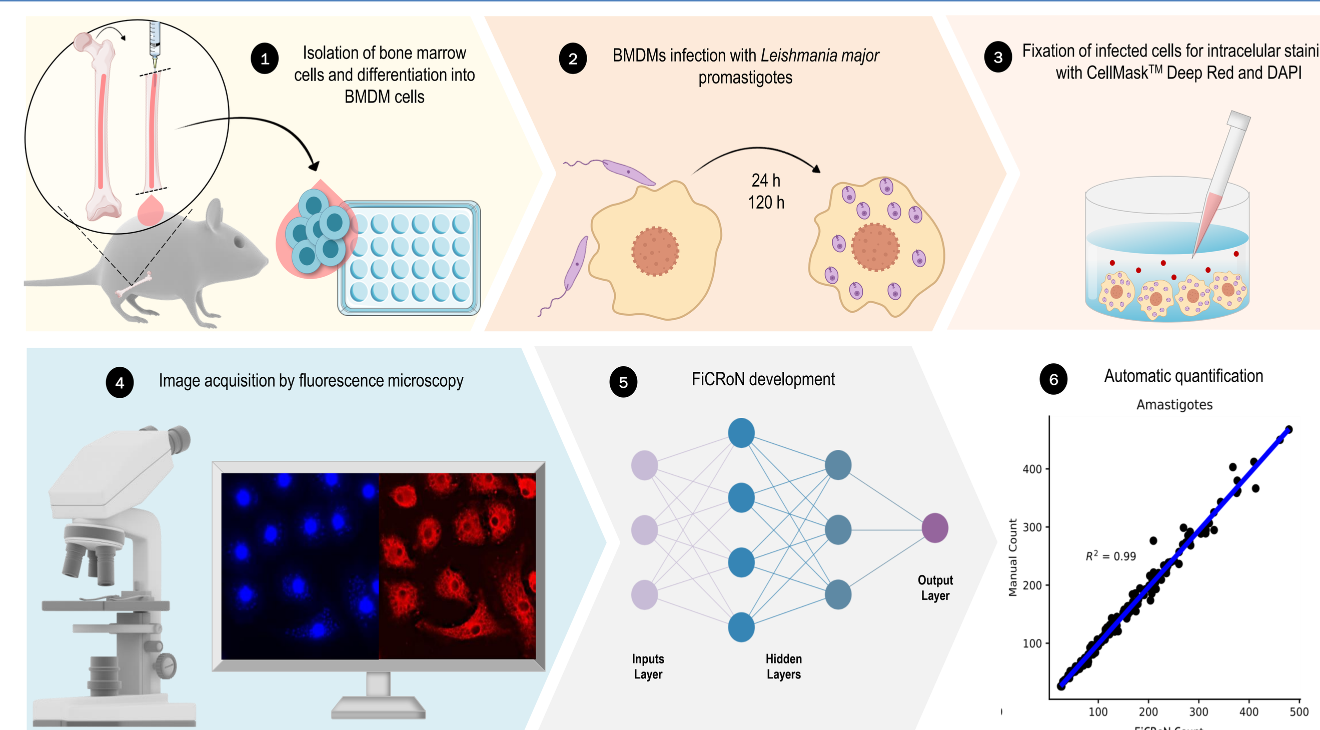


Crowd counting, density maps

3D reconstruction.
Leishmania infected macrophage

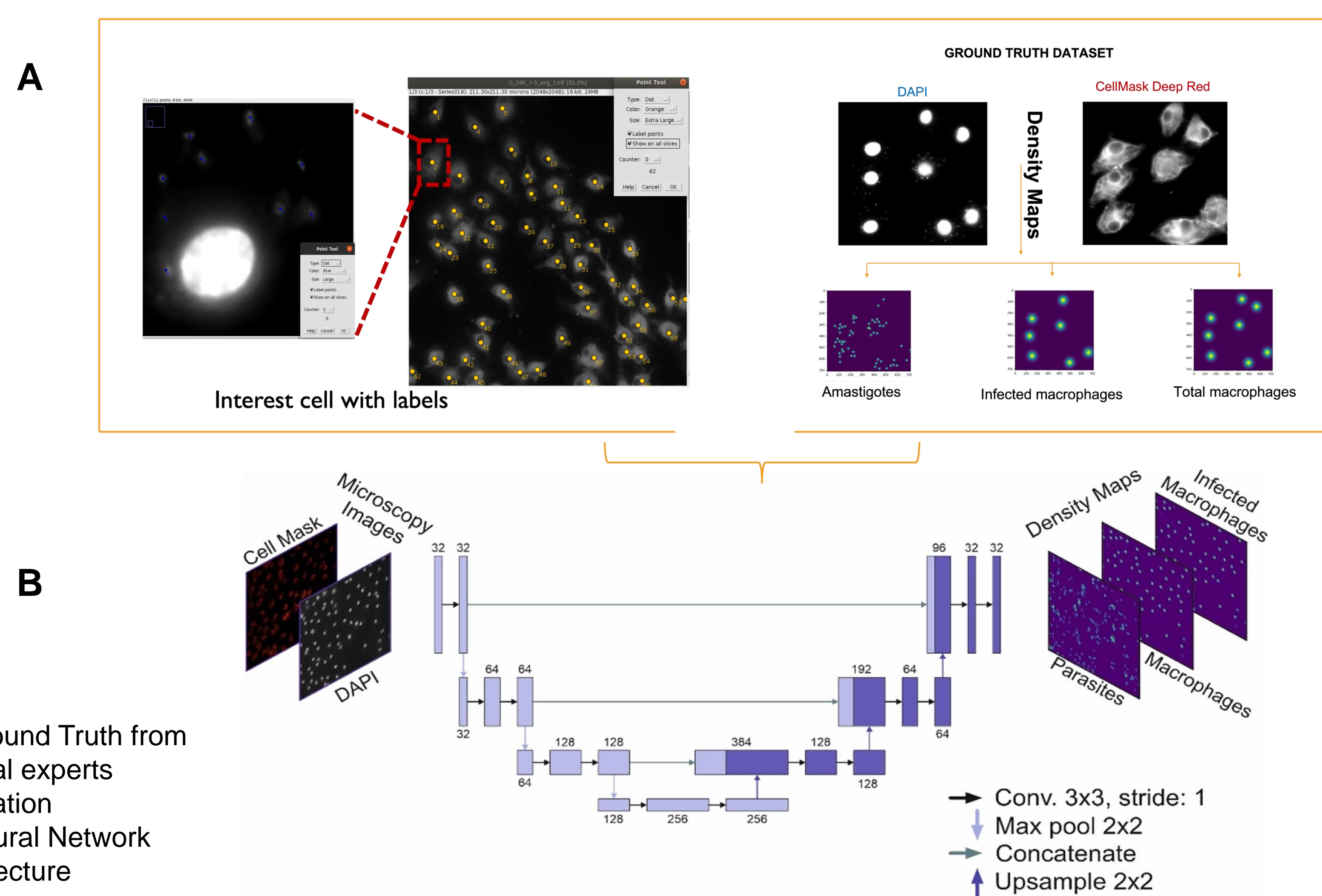
SangÅ *et al.*, IEEE Access, 2019.

2. Methodology



3. Design of the FiCRoN method

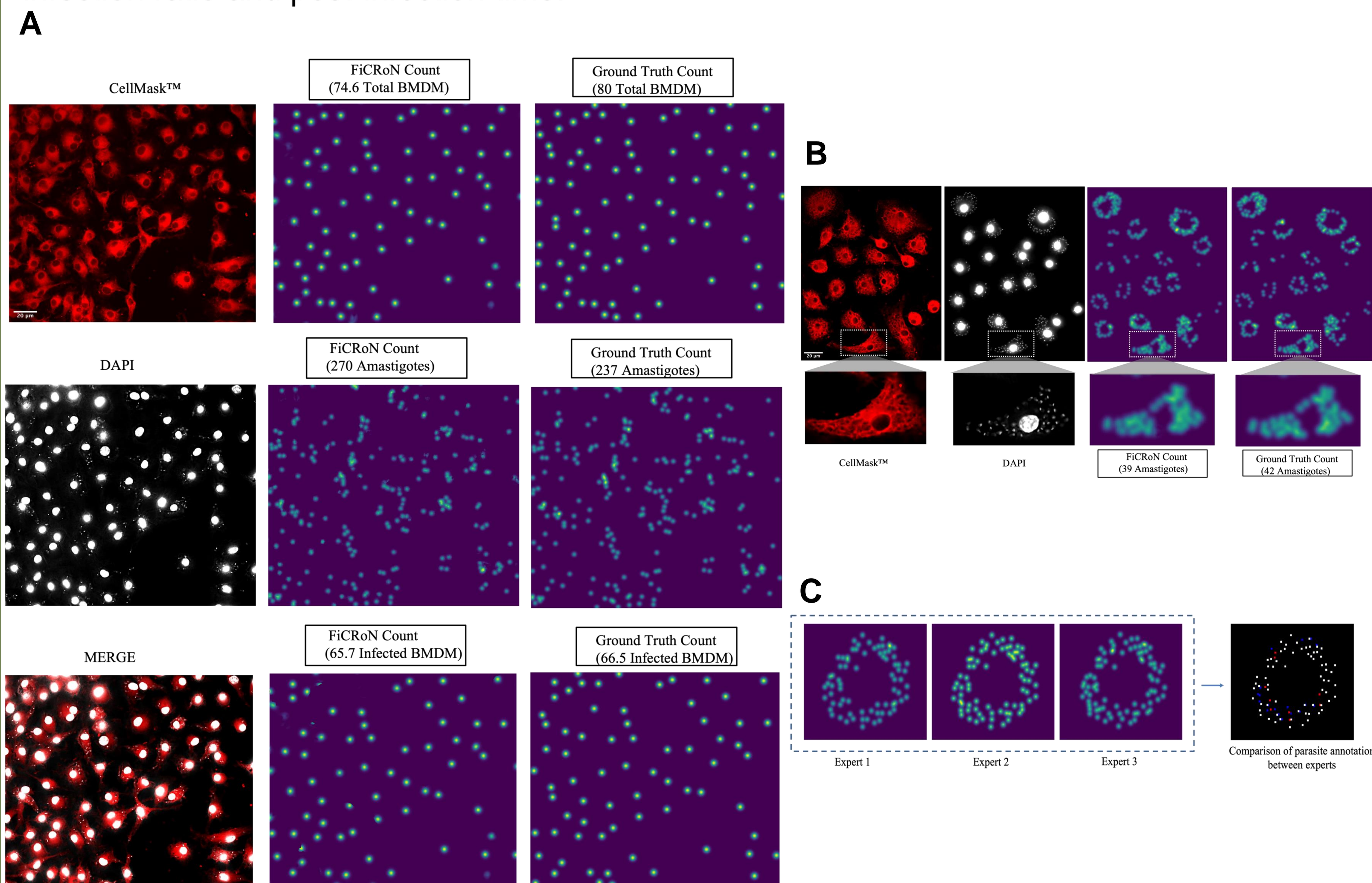
Our proposed method consists in the quantification of three different cells class using Deep neural network regression and classification. This network learns to generate pixel-by-pixel images whose result is the density map of each cell of interest, so that the total number of cells per category is obtained by integrating its corresponding density map.



A. Ground Truth from manual experts annotation
B. Neural Network Architecture

4. Training and Validation Dataset

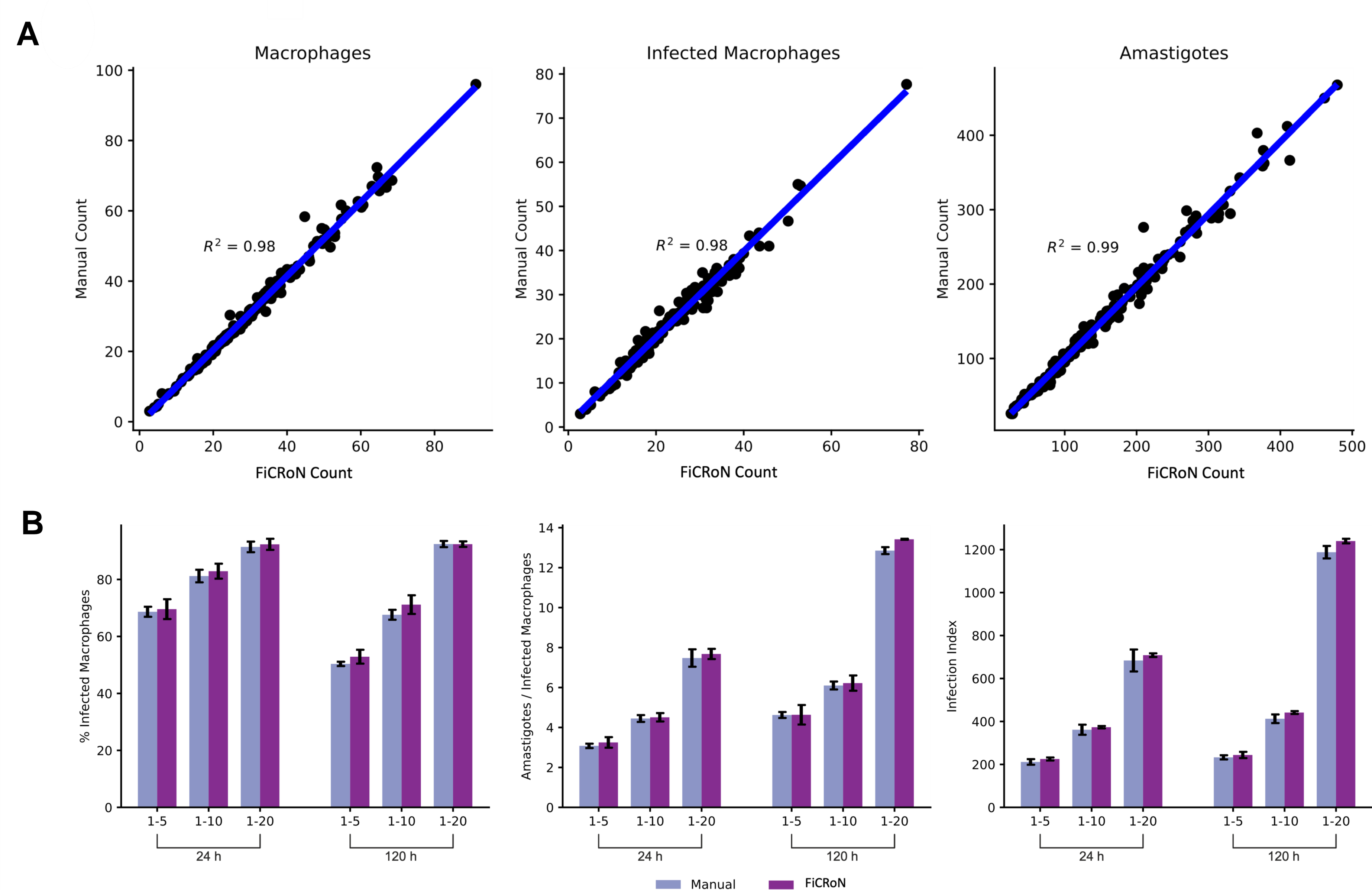
This data subset contains three cell category, total macrophages, infected macrophages and *Leishmania* amastigotes. In order to have diversity in the parasite burden, 6 data subsets of fluorescence microscopy images were generated. A data subset was obtained for each MOI 1:5, 1:10, 1:20 at 24 and 120 h. In this type of infection processes are frequently obtained image with a high content of macrophages and amastigotes, more than 30 macrophages and 100 amastigotes can be visualized in one image, depending on the infection ratio and post-infection time.



Fluorescence Microscopy Validation Images and Density Maps. A. Infected macrophages with *L. major* with MOI 1:10 at 120hrs post-infection. To determine cell-cell borders, macrophages cytoplasm were stained with CellMask™ Deep Red and macrophages nuclei and intracellular parasites were stained with DAPI. **B.** Image crop of infected macrophages with MOI 1:20 at 120hrs post-infection. **C.** Comparison of parasite annotation between experts. Matching parasites with all experts annotation (white dots), matching parasites with two experts annotation (blue dots) and parasites annotated by one expert (red dots).

5. Testing and Application

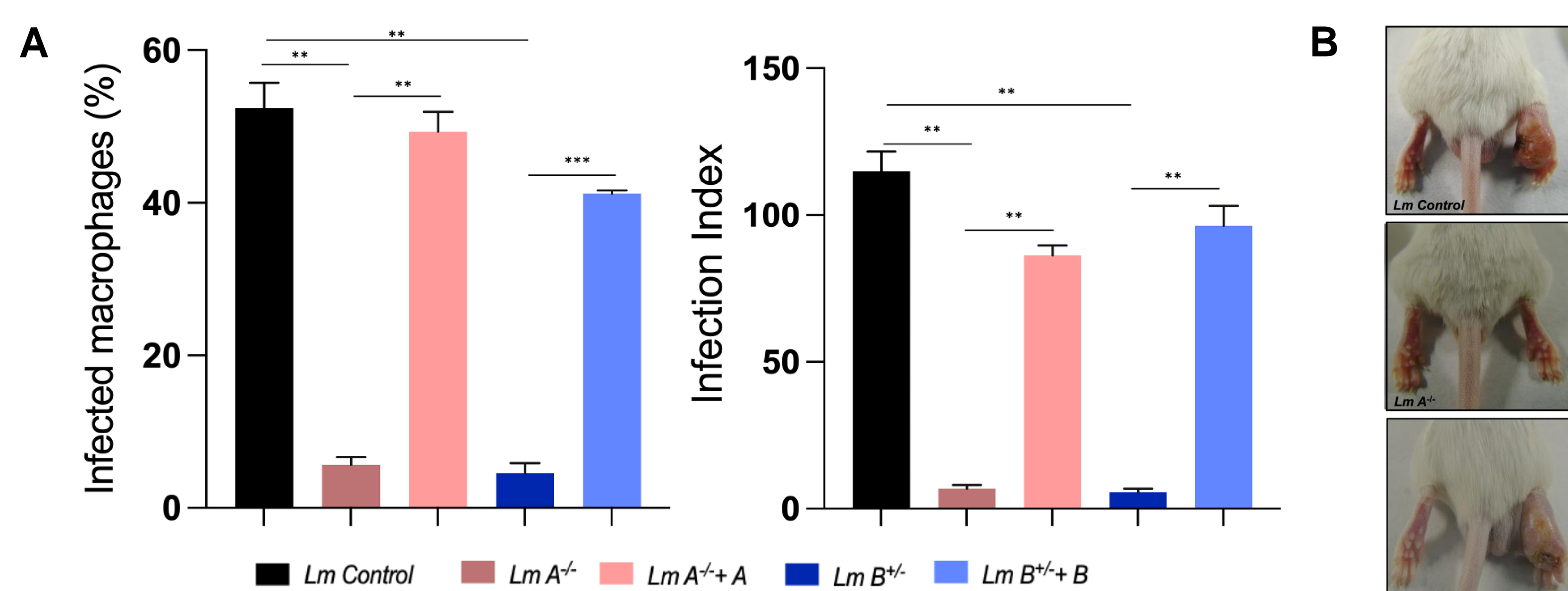
To determine the final performance of the neural network a sub dataset test was obtained. The number of infected and uninfected macrophages and amastigotes within infected cells was quantified for each MOI (1:5, 1:10 and 1:20) and post-infection times (24h and 120 h). The performance of the algorithm showed a high degree of correlation between them compared to manual counting.



Counting performance of deep learning method. A. Linear correlation between automatic count and manual count. Number of total macrophages (left), number of infected macrophages (middle) and number of amastigotes (right). **B.** Parasite burden measurement metrics were also calculated using the manual and automatic methods, % Infected macrophages (left), Amastigotes/infected macrophage (middle) and Infection index (right).

Application of the FiCRoN method in *Leishmania* Knockout lines

As a tool for use, the FiCRoN method was implemented in the determination of the parasite burden of different lines of *Leishmania* of scientific interest. We generated knockout parasites, deleting one (single knockout, *Lm* $B^{+/-}$) or both alleles (double knockout, *Lm* $A^{-/-}$) using CRISPR-Cas9 technology. These results were correlated with virulence capacity in a *in vivo* model of cutaneous leishmaniasis.



Automatic quantification of Parasite Burden in macrophages infected with *Leishmania* mutant lines. A. % Infected macrophages (left) and Infection index (right) were calculated using FiCRoN, using control (*Lm Control*), double knockout (*Lm* $A^{-/-}$) and complemented (*Lm* $A^{-/-}$*+A*) lines. **p < 0.05; ***p < 0.005. **B.** Representative images of inoculated footpads of mice infected with the same *Leishmania* strains.

Conclusion: Our FiCRoN method is an innovative artificial intelligence-based image analysis designed to provide a fast and accurate quantification of *Leishmania* infection in macrophages, potentially useful for other intracellular parasites.