

Counting or Localizing? Evaluating Cell Counting and Detection in Microscopy Images

Luca Ciampi^a, Fabio Carrara^b, Giuseppe Amato^c and Claudio Gennaro^d

Institute of Information Science and Technologies, National Research Council, Pisa, Italy

Keywords: Automatic Cell Counting, Biomedical Image Analysis, Deep Learning, Deep Learning for Visual Understanding, Convolutional Neural Networks, Counting Objects in Images, Visual Counting.

Abstract: Image-based automatic cell counting is an essential yet challenging task, crucial for the diagnosing of many diseases. Current solutions rely on Convolutional Neural Networks and provide astonishing results. However, their performance is often measured only considering counting errors, which can lead to masked mistaken estimations; a low counting error can be obtained with a high but equal number of false positives and false negatives. Consequently, it is hard to determine which solution truly performs best. In this work, we investigate three general counting approaches that have been successfully adopted in the literature for counting several different categories of objects. Through an experimental evaluation over three public collections of microscopy images containing marked cells, we assess not only their counting performance compared to several state-of-the-art methods but also their ability to correctly localize the counted cells. We show that commonly adopted counting metrics do not always agree with the localization performance of the tested models, and thus we suggest integrating the proposed evaluation protocol when developing novel cell counting solutions.

1 INTRODUCTION

Microscopy medical images analysis comprises several challenging Computer Vision problems involving a wide variety of tasks. Among them, cell localization (Lugagne et al., 2019) and counting (Falk et al., 2018) are essential steps for basic research, like disease diagnosis via the evaluation of cell growth kinetics, the estimation of cytotoxicity (i.e., the quality of being toxic to cells) (Kotoura et al., 1985), the quantification of perineuronal nets (Fawcett et al., 2019), the discovery of the role of particular genes in cell biology, microbiology, and immunology (Zhang et al., 2015), and many more. Manual cell counting is still conducted in many laboratories, often with the aid of a hemocytometer and its variants, which has been commonly used due to its low cost and versatility (Johnston, 2010). However, the procedure is time-consuming and error-prone, being subject to inter-user variation depending on the degree of expertise of the analyst (Altman et al., 1993). Therefore, there is a need to

count cells automatically to facilitate this tedious and challenging task.

Recently, several vision models (mostly based on Convolutional Neural Networks) have been successfully adopted to count cells and other biological structures from microscopy images. However, the performance of these techniques is often measured only considering the counting errors occurring at inference time (i.e., the difference between the predicted and the actual cell numbers), which often leads to masked mistaken estimations. Indeed, counting errors do not take into account *where* the cells have been localized in the images and, consequently, counting models might achieve low values of errors while providing wrong predictions (e.g., a high number of false positives and false negatives). Therefore, it is hard to perform a fair comparison between the different state-of-the-art cell counting approaches to determine which performs best.

In this work, we investigate three baseline solutions belonging to the three main counting methodologies — a *segmentation-based* approach, a *localization-based* approach, and a *count-density estimation* approach — that have been successfully exploited for counting several different categories of objects, such as people and vehicles, and that repre-

^a <https://orcid.org/0000-0002-6985-0439>

^b <https://orcid.org/0000-0001-5014-5089>

^c <https://orcid.org/0000-0003-0171-4315>

^d <https://orcid.org/0000-0002-3715-149X>

sent the conceptual basis also for the cell counting techniques. We conduct experiments on three public datasets containing different cell types and characterized by distinct peculiarities. In addition to comparing the performance of investigated methods against state-of-the-art cell counters using established counting evaluation metrics, we also measure the ability of the models to localize the counted cells correctly. Specifically, we adopt two additional metrics; a) the Grid Average Mean absolute Error (GAME) metric, a hybrid metric that simultaneously considers errors in the object count and in their coarse location, and b) the mean Average Precision (mAP), that summarizes the cell precise localization performance. We show that commonly adopted *counting* metrics (like mean absolute error) do not always agree with the *localization* performance of the tested models, and thus we suggest measuring both whenever possible to facilitate the practitioner in picking the most suitable solution.

We organize the paper as follows. We review related work in Section 2. In Section 3, we describe the datasets used for our experiments. Section 4 describes the investigated methodologies, while Section 5 outlines the performed experiments and the obtained results. Finally, Section 6 concludes the paper. The code and the trained models are publicly available at https://github.com/ciampluca/counting-perineuronal_nets/tree/visapp-counting-cells.

2 RELATED WORKS

This section reviews some works concerning the counting task in its generality and specifically tailored to estimating the number of cells in microscopy images.

Visual Counting. The goal of the visual counting task is to estimate the number of object instances in still images or video frames (Lempitsky and Zisserman, 2010). Due to its interdisciplinary and widespread applicability to many real-world applications, like calculating the number of people present at an event (Boominathan et al., 2016), evaluating the number of vehicles in urban scenarios (Ciampi et al., 2021a), or counting animals in ecological surveys (Arteta et al., 2016b), visual counting has recently drawn the attention of researchers. Current solutions address this task as a supervised deep learning-based process. They fall into two main categories: *counting by detection* (Amato et al., 2019; Amato et al., 2018; Laradji et al., 2018; Ciampi et al., 2018) that requires prior detection or segmentation of

the single instances of objects, and *counting by regression* (Oñoro-Rubio and López-Sastre, 2016; Li et al., 2018; Ciampi et al., 2020; Ciampi et al., 2021b) that instead tries to establish a direct mapping between the image features and the number of objects in the scene, either directly or via the estimation of a density map (i.e., a continuous-valued function). Regression techniques have demonstrated superior performance in crowded scenarios where the objects' instances are sometimes not well visible due to occlusions and clumps. However, they cannot precisely localize the objects present in the scene, eventually providing only a coarse position of the area in which they are distributed.

Microscope Cell Counting. Because of its paramount importance, several cell counting deep learning-based methods have been proposed in the last years. They belong to both the detection-based and the regression-based approaches, each having the advantages and the drawbacks already discussed above. A relevant example belonging to the former category is (Paulauskaite-Taraseviciene et al., 2019), where authors exploited the popular Mask R-CNN (He et al., 2017) instance segmentation framework to detect overlapping cells. On the other hand, a notable regression-based work is (Aich and Stavness, 2018), where the authors regulated activation maps from the final convolutional layer of the network by exploiting coarse ground-truth activation maps generated from simple dot annotations. Authors in (Xie et al., 2016), instead, introduced a CNN-based regression approach that maps the image features with an associated density map, providing also a coarse localization of the cells by finding its peak values. Another example is represented by (Segui et al., 2015), where the authors proposed a regression-based technique and explored the features that are learned to understand their underlying representation. In (Cohen et al., 2017), another regression-based deep neural network architecture (named Count-ception) is presented, inspired by the Inception family (Szegedy et al., 2015). More, in (Guo et al., 2021), another density-based deep network framework designed to solve the cell counting task is introduced. Specifically, the authors propose SAU-Net, extending the segmentation network U-Net (Ronneberger et al., 2015) with a Self-Attention module. Finally, in (He et al., 2021) the authors exploited auxiliary CNNs to assist the training of the intermediate layers of a density regressor. Hybrid strategies have also been devised to deal with densely concentrated cells but still generating individual cell detections, such as (Falk et al., 2018; Xie et al.,

2018). These approaches first generate intermediate maps that indicate the likelihood of each pixel being the center of a cell in the image. Then, they convert these maps into detections by applying some form of Non-Maximum Suppression (NMS).

Most of these works measure the counting performance by computing the error between the predicted and the actual cell number, hiding potentially mistaken localization. In this work, we consider three general counting approaches on which cell-specific techniques rely, and we also evaluate the quality of the produced detections.

3 DATASETS

In this section, we describe the datasets employed in this work, summarized in Table 1; in particular, we consider three publicly available collections of microscopy images widely used in the context of the cell counting task, presenting different peculiarities and challenges.

3.1 VGG Cells Dataset

The *VGG Cells* dataset, introduced in (Lempitsky and Zisserman, 2010), comprises 200 RGB highly-realistic synthetic emulations of fluorescence microscopy images of bacterial cells. Images have a fixed size of $256 \times 256 \times 3$ pixels, and the cells are clustered in specific regions and occluded with each other. It is worth noting that the annotation procedure is performed automatically and so labels are free of errors. We show a sample of this dataset in Figure 1.

3.2 MBM Cells Dataset

The *Modified Bone Marrow (MBM) Cells* has been initially collected by the authors of (Kainz et al., 2015) from 11 RGB microscopy images (having a fixed size of $1200 \times 1200 \times 3$ pixels) of the human bone marrow tissues pertaining to 8 different patients. The marked cells belonging to this dataset have a significant shape variance; furthermore, non-homogeneous tissue background makes their localization more difficult. In a subsequent work (Cohen et al., 2017), the authors divided each image into four patches of $600 \times 600 \times 3$ pixels, for a total of 44 images. A sample of this dataset is reported in Figure 1.

3.3 Nuclei Cells Dataset

This dataset has been presented in (Sirinukunwattana et al., 2016) and comprises 100 RGB microscopy

H&E stained histology images of colorectal adenocarcinomas having a common size of $500 \times 500 \times 3$. The images refer to 9 different patients. They have been cropped from non-overlapping areas representing a variety of tissue appearances from normal and malignant regions. Still, they also comprise areas with artifacts, over-staining, and failed autofocussing to simulate realistic outliers. Another peculiarity of this dataset is that the nuclei of the cells belong to four different categories, presenting different visual characteristics; some experts have manually annotated them by putting a dot over the centroids of each biological structure for a total of 29,756 nuclei marked. In the following, we refer to this dataset as *Nuclei Cells* dataset. We report a sample of this dataset in Figure 1.

4 METHOD

We assume to have a labeled collection of N microscopy images $\mathcal{X} = \{(I_1, \hat{L}_1), \dots, (I_N, \hat{L}_N)\}$, where \hat{L}_i is the set of 2D-point annotations associated to the i -th image I_i . Each image has been manually annotated by a human expert, and the annotations are in the form of dots, i.e., coordinates localizing the centroids of the cells present in the region of interest, as is usually the case in the counting task.

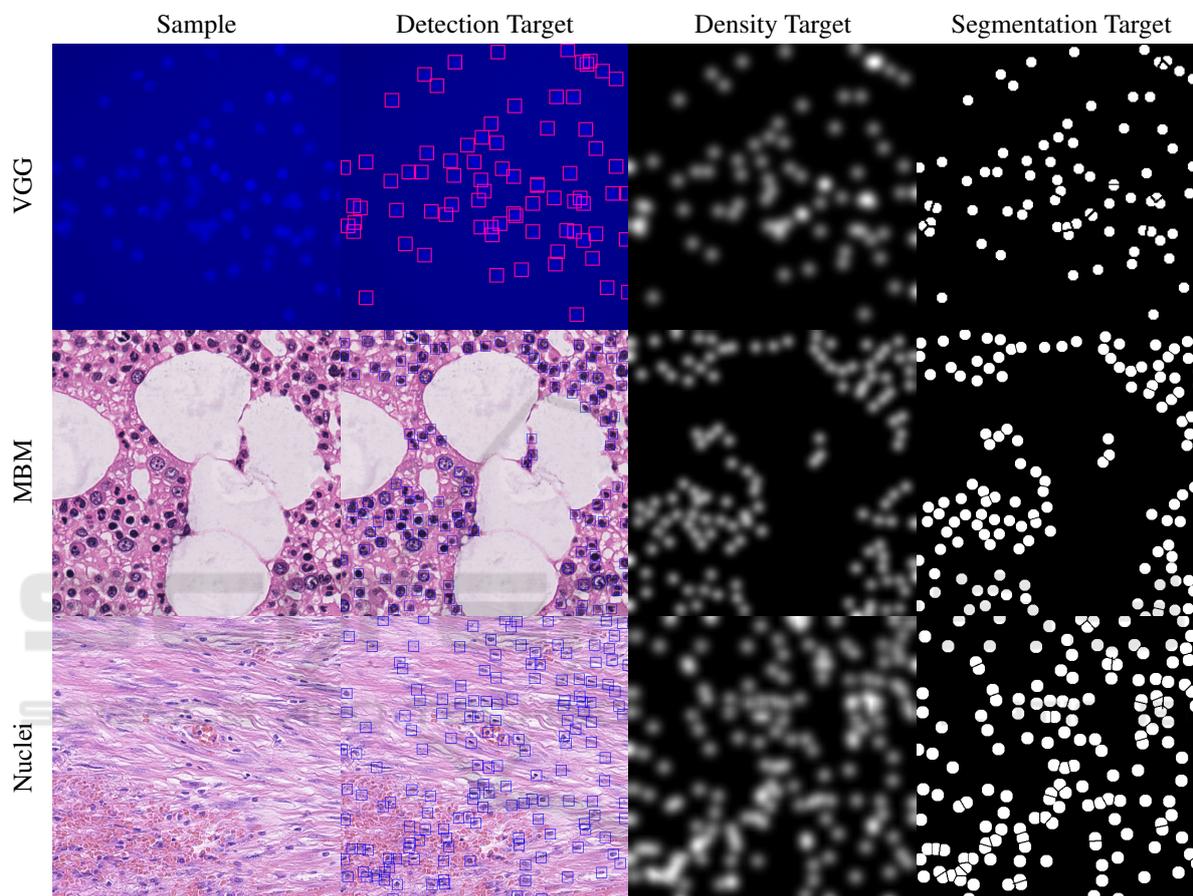
We define a localization model f_θ as a Deep Learning-based algorithm that takes as input an image I and produces as output an associated set of coordinates $L = \{p_1, \dots, p_C \mid p_j \in \mathbb{R}^2\}$ localizing the centroids of the cells to be counted. This model is trained using location data \mathcal{X} and can be implemented following several different strategies; here, we test three successful approaches from the literature, that are *segmentation*, *detection*, and *density estimation*, described below.

4.1 Foreground/Background Segmentation

Proposed by (Falk et al., 2018), in this approach we locate cells on the basis of a binary segmentation map $S \in \{0, 1\}^{H \times W}$ where ones represent pixels of objects of interest, while zeros are considered background. Each connected component in the segmentation map represents a single object; the positions of the objects L are set to the coordinate of the centroids of the connected components. As the implementation of the model f_θ , we adopt the original U-Net architecture (Ronneberger et al., 2015) commonly used in segmentation tasks. The model is trained to produce a real-valued segmentation map $\hat{S} = f_\theta(I) \in [0, 1]^{H \times W}$

Table 1: **Summary of datasets.** We show the different peculiarities that characterize the three datasets exploited in this work.

Dataset	N.Img	Size	N.Obj	Objs/Img
VGG (Lempitsky and Zisserman, 2010)	200	256×256	35,192	176 ± 61
MBM (Kainz et al., 2015; Cohen et al., 2017)	44	600×600	5,553	126 ± 33
Nuclei (Sirinukunwattana et al., 2016)	100	500×500	29,756	297 ± 218

Figure 1: **Samples and Targets.** We show a dataset sample (1st column) and the corresponding targets used when training i) the detection-based method *FRCNN* (2nd column), ii) the density-based method *D-CSRNet* (3rd column), and iii) the segmentation-based method *S-UNet* (4th column).

that is then thresholded to obtain S . The target segmentation maps are generated drawing discs at the annotated positions and carefully separating overlapping discs with a background ridge (see the fourth column of Figure 1 for examples of targets). We minimize the weighted binary cross-entropy between pixels of the output and target maps as specified in (Falk et al., 2018); more important pixels (near ridges and foreground objects) are given an increased weight in the total loss computation. We will refer to this approach as *S-UNet*.

4.2 Bounding Box Regression

For this approach, we employ the standard Faster-RCNN detector (Ren et al., 2017). This deep neural network takes images as input and produces a list of bounding boxes localizing the objects as output. The detection pipeline follows the two-stage paradigm. In the first stage, the network generates a bunch of region proposals likely to contain objects, exploiting a set of *anchors* (i.e., pre-defined boxes) that are sliced over the image; in the second stage, these priors are refined and, for each of them, a score is assigned expressing the likelihood to really containing the object. We consider the centers of the final boxes as the lo-

calization of the entities we want to consider. We produce the targets by generating squared bounding boxes centered in the dot-annotated data and having fixed sides, again, depending on the typical object size in the dataset. A sample of a target is shown in the second column of Figure 1. We implement f_θ as a Faster-RCNN network with a Feature Pyramid Network module and a ResNet-50 backbone. From now on, we will refer to this method as *FRCNN*.

4.3 Density Estimation

We also account for density-estimation approaches that have shown superior counting performances in very “crowded” scenarios. In this case, the goal is to learn a regression between the features of an input image having height H and width W to a density map $D = f_\theta(I) \in \mathbb{R}^{H \times W}$. The notion of density map is close to the physical/mathematical notion of density; specifically, each pixel of D corresponds to the *quantity* of the objects present at that precise location. The number of the objects n present in an image sub-region $P \subseteq I$ is estimated by summing up pixel values in the region of interest, i.e., $n = \sum_{p \in P} D_p$. Although these approaches are not suited for precisely localize objects, a coarse localization can be obtained by analyzing the estimated density map, in particular by finding the top- n maximum local peaks of it, as already done in (Xie et al., 2016). We train the model by minimizing the mean squared error loss between target and predicted density maps. Following previous works, we generate the target density maps by superimposing Gaussian kernels G_σ centered in the dot-annotated locations; the spread parameter σ is fixed, and it has been estimated depending on the typical object size in the considered dataset. We show an example of a target density map in the third column of Figure 1. We implement f_θ exploiting the Congested Scene Recognition Network (CSRNet), proposed in (Li et al., 2018), a CNN for accurate density estimation of congested scenes, comprising two major components. Specifically, it uses a modified version of the popular VGG-16 network (Simonyan and Zisserman, 2015) to extract the image features; stacked upon this, the authors built a back-end composed of dilated convolutional (Yu and Koltun, 2016) layers to extract deeper information of saliency and, at the same time, maintain the output resolution. We will refer to this method as *D-CSRNet*.

5 EXPERIMENTS AND RESULTS

In this section, we describe the experiments performed to validate our approach and discuss the obtained results. First, we evaluate the three adopted general counting solutions, i.e., the segmentation-based S-UNet, the detection-based FRCNN, and the density-based D-CSRNet approaches, over the three standard cell counting benchmarks described above to verify that the obtained counting errors are comparable with the ones provided by state-of-the-art cell-specific counting methods. Then, we perform additional experiments evaluating the quality of the *localization* of the cells, an aspect that is not taken into account by counting metrics.

5.1 Comparison with the State-of-the-Art

We evaluate the three adopted counting methodologies over the VGG Cells, the MBM Cells, and the Nuclei Cells counting benchmarks described in Section 3, and we compare their performances with other state-of-the-art approaches. For the VGG Cells and the MBM Cells datasets, we follow the evaluation protocol introduced by (Lempitsky and Zisserman, 2010) and adopted by most subsequent works. Specifically, we consider a testing subset fixed for all the experiments (100 and 10 images for VGG Cells and MBM Cells, respectively) and training and validation subsets of varying size (N images for each subset) to simulate lower or higher numbers of labeled examples. This evaluation protocol simulates the real scenario in which scientists often have a significant variance regarding the number of available microscopy images. Following previous work, we set N to 16, 32, and 50 for VGG Cells and to 5, 10, 15 for MBM Cells. Concerning the Nuclei Cells dataset, we instead use two-fold cross-validation, with 50 images for testing, according to (Sirinukunwattana et al., 2016) and subsequent works. Following standard counting benchmarks, we use the Mean Absolute Error (MAE) to measure the counting performance. Specifically, it is defined as:

$$\text{MAE} = \frac{1}{N} \sum_{n=1}^N \left| c_{\text{gt}}^n - c_{\text{pred}}^n \right|, \quad (1)$$

where N is the number of test images, c_{gt}^n is the actual count (i.e., the ground truth), and c_{pred}^n is the predicted count of the n -th image. For the VGG Cells and the MBM Cells, we repeat the experiment 10 times, randomly sampling ten different splits for each configuration, and we report the mean and standard

deviation of the MAE computed between the different runs. On the other hand, concerning the Nuclei dataset, we report the mean and the standard deviation of the MAE calculated between the 100 images comprising the two test splits.

Table 2 reports the obtained results. The density-based solution performs best among the VGG Cells dataset and, more strongly, with the Nuclei Cells dataset, comparably to the state of the art. The other two adopted methods, i.e., the segmentation-based S-UNet and the detection-based FRCNN, show larger errors, according to their intrinsic limitations when employed in highly “crowded” scenarios with occluded objects like the VGG Cells dataset and, especially, the Nuclei Cells dataset. On the other hand, considering the MBM Cells dataset, characterized by challenges more related to the object shape variations, all the approaches show competitive results, in some cases also outperforming state-of-the-art solutions.

5.2 Localization Analysis

Although the MAE is a fair metric for establishing a comparative in terms of counting, it can often lead to masking erroneous estimations. The reason is that the MAE does not take into account *where* the estimations have been done in the images. In other words, the MAE does not capture localization errors; models might achieve low values of MAE while providing wrong predictions (e.g., a high number of false positives and false negatives in detection-based techniques, or a bad allocation of density values in predicted maps of density-based methods). Hence, picking up the best counting model basing the decision only on the MAE metric can lead to blunders.

In this section, we conduct experiments to assess the ability of the three adopted solutions to localize the counted cells correctly. Specifically, we consider two additional metrics described in the following paragraphs.

Grid Average Mean absolute Error (GAME). (Guerrero-Gómez-Olmedo et al., 2015) is a hybrid metric that simultaneously considers the object count and the estimated locations of the cells. Specifically, it is computed by sub-dividing the image in 4^L non-overlapping regions and summing the MAE computed in each of these sub-regions. Formally:

$$GAME(L) = \frac{1}{N} \sum_{n=1}^N \left(\sum_{l=1}^{4^L} |c_{gt}^l - c_{pred}^l| \right), \quad (2)$$

where N is the total number of test images, c_{pred}^l is the estimated count in a region l of the n -th image, and

c_{gt}^l is the ground truth for the same region in the same image. The higher L , the more restrictive the GAME metric will be. Note that the MAE can be obtained as a particular case of the GAME when $L = 0$.

Mean Average Precision (mAP). is an established metric for the localization performances of object detectors. We compute the average precision for an image as follows. i) We assign a score to each detected cell in the image. Detection scores are obtained differently for the three tested methods. For the S-UNet model, the detection score of an object is set to the value of the predicted segmentation map \hat{S} at the location of the centroid of the corresponding connected component. For D-CSRNet, the location of an object is set to a local peak in the predicted density map, and its score is set to the value at its location. For FRCNN, the detection score is already part of the output of the Faster-RCNN model. ii) We filter out weak detections using a threshold on the detection scores. iii) We match the filtered detections with the ground-truth object positions using the Hungarian algorithm with a constraint on the maximum accepted displacement in pixels between predicted and real locations; once matches are found, we obtain the number of true positives (matched detection and ground-truth pairs), false positives (unmatched detections), and false negatives (unmatched ground-truth positions) locations. iv) We repeat these steps for several threshold values to obtain the precision-recall curve and the average precision (i.e., the area under the curve).

In Table 3, we report the MAE, the GAME, and the mAP metrics for all the tested solutions and the adopted datasets. Here, we consider only the splits having N to 50 and 15 for the VGG and the MBM datasets, respectively, and the same two-fold cross-validation with 50 images for testing concerning the Nuclei dataset. Note that the density-based solution D-CSRNet shines in the Nuclei benchmark where very dense regions of overlapped cells are common and strain non-density solutions, obtaining the best counting metrics (MAE, GAME) among the tested models. However, the denser the cells in the benchmark, the less the density-based solution can recover the exact locations of the counted cells, thus achieving lower mAP values. On the other hand, the detection-based solution FRCNN performs sufficiently well only when counting cells in the less crowded MBM and VGG benchmarks. Still, it is able to recover the exact position of more counted cells, as can be seen from the higher mAP values obtained. Last, the segmentation-based model sits in the middle of these two extremes, providing intermediate counting and localization performance.

Table 2: **Comparison on Standard Benchmarks.** For *VGG* and *MBM* datasets, we vary the training and validation subsets (N images for each subset), repeating the experiments 10 times. For *Nuclei*, we perform 2-fold cross-validation ($N = 50$ images per fold). Mean \pm st.dev. of MAE is reported.

VGG Cells (Lempitsky and Zisserman, 2010). (200 images in total - 100 test images)

Method	N = 16	N = 32	N = 50
(Arteta et al., 2016a)	N/A	5.06 \pm 0.2	N/A
GMN (Lu et al., 2019)	N/A	3.6 \pm 0.3	N/A
(Lempitsky and Zisserman, 2010)	3.8 \pm 0.2	3.5 \pm 0.2	N/A
VGG-GAP-HR (Aich and Stavness, 2018) *	N/A	2.95**	2.67
SAU-Net (Guo et al., 2021)	N/A	N/A	2.6 \pm 0.4 \dagger
FCRN-A (Xie et al., 2016)	3.4 \pm 0.2	2.9 \pm 0.2	2.9 \pm 0.2 \ddagger
Count-Ception (Cohen et al., 2017)	2.9 \pm 0.5	2.4 \pm 0.4	2.3 \pm 0.4
CCF (Jiang and Yu, 2020)	2.8 \pm 0.1	2.6 \pm 0.1	2.6 \pm 0.1
C-FCRN+Aux (He et al., 2021)		2.3 \pm 2.2 $\$$	
S-UNet (Falk et al., 2018)	8.3 \pm 2.3	5.6 \pm 1.1	4.5 \pm 0.5
D-CSRNet (Li et al., 2018)	4.0 \pm 0.2	3.2 \pm 0.2	3.0 \pm 0.1
FRCNN (Ren et al., 2017)	9.3 \pm 0.7	8.2 \pm 0.6	7.4 \pm 1.0

* They did not report standard deviation. ** They used a validation subset of $100 - N$ images. \dagger They did not use a test subset, but only a $100 - N$ images validation subset. \ddagger Reported in their work as $N = 64$. $\$$ They used a 5-fold cross validation-based evaluation protocol considering the whole dataset.

MBM Cells (Kainz et al., 2015; Cohen et al., 2017). (44 images in total - 10 test images)

Method	N = 5	N = 10	N = 15
(Xie et al., 2018)		36.3 \pm 19.4 $\$$	
FCRN-A (Xie et al., 2016)	28.9 \pm 22.6	22.2 \pm 11.6	21.3 \pm 9.4
(Marsden et al., 2018) *	23.6 \pm 4.6	21.5 \pm 4.2	20.5 \pm 3.5
Count-Ception (Cohen et al., 2017)	12.6 \pm 3.0	10.7 \pm 2.5	8.8 \pm 2.3
CCF (Jiang and Yu, 2020) *	9.3 \pm 1.4	8.9 \pm 0.9	8.6 \pm 0.3
C-FCRN+Aux (He et al., 2021)		6.5 \pm 5.2 **	
SAU-Net (Guo et al., 2021)	N/A	N/A	5.7 \pm 1.2 \dagger
S-UNet (Falk et al., 2018)	9.0 \pm 1.9	7.0 \pm 1.6	6.7 \pm 2.5
D-CSRNet (Li et al., 2018)	10.8 \pm 2.5	8.0 \pm 1.3	7.0 \pm 1.3
FRCNN (Ren et al., 2017)	8.8 \pm 1.4	9.9 \pm 1.5	8.3 \pm 1.9

* They used 14 test images. ** They used a 5-fold cross validation-based evaluation protocol considering the whole dataset. \dagger They did not use a test subset, but only a $44 - N$ images validation subset. $\$$ They used a train/test split of 8/3 using full-size images.

Nuclei Cells (Sirinukunwattana et al., 2016). (100 images in total - 50 test images)

Method	N = 50
DeepFeat (Segui et al., 2015)	71.8 \pm 51.4
(Lempitsky and Zisserman, 2010)	51.4 \pm 39.8
StructRegNet (Xie et al., 2018)	45.9 \pm 47.9
FCRN-A (Xie et al., 2016)	42.5 \pm 33.5
Count-Ception (Cohen et al., 2017)	34.1 \pm 29.0
C-FCRN+Aux (He et al., 2021)	29.3 \pm 25.4
S-UNet (Falk et al., 2018)	62.4 \pm 55.4
D-CSRNet (Li et al., 2018)	37.3 \pm 41.0
FRCNN (Ren et al., 2017)	96.5 \pm 128.0

Table 3: **Counting and Localization Performance.** The MAE measures global counting performance independently of localization. The mAP summarizes localization performances in terms of precision and recall of localized cells. The GAME(L) measure counting performance while being aware of the location of cells; the higher L , the more localization errors are penalized. Regarding the VGG and the MBM datasets, we consider the splits having N to 50 and 15, respectively.

VGG Cells (Lempitsky and Zisserman, 2010). (200 images in total - 100 test images)						
Method	MAE ↓	GAME(L) ↓				mAP (%) ↑
		$L = 1$	$L = 2$	$L = 3$	$L = 4$	
S-UNet	4.5 ± 0.5	7.7 ± 1.3	12.8 ± 1.5	21.6 ± 2.4	38.0 ± 4.1	75.3 ± 15.8
D-CSRNet	3.0 ± 0.1	6.5 ± 0.2	11.3 ± 0.4	18.9 ± 0.6	28.7 ± 1.0	43.2 ± 1.6
FRCNN	7.4 ± 1.0	11.1 ± 0.9	18.3 ± 1.3	29.7 ± 2.0	43.3 ± 3.2	93.3 ± 0.6

MBM Cells (Kainz et al., 2015; Cohen et al., 2017). (44 images in total - 10 test images)						
Method	MAE ↓	GAME(L) ↓				mAP (%) ↑
		$L = 1$	$L = 2$	$L = 3$	$L = 4$	
S-UNet	6.7 ± 2.5	10.4 ± 2.5	17.3 ± 1.9	27.6 ± 2.0	40.9 ± 3.1	53.5 ± 5.3
D-CSRNet	7.0 ± 1.3	10.8 ± 1.2	16.7 ± 1.3	27.0 ± 1.6	41.5 ± 2.2	67.9 ± 1.2
FRCNN	8.3 ± 1.9	12.7 ± 2.4	20.4 ± 3.9	32.5 ± 4.7	47.2 ± 8.7	87.4 ± 1.9

Nuclei Cells (Sirinukunwattana et al., 2016). (100 images in total - 50 test images)						
Method	MAE ↓	GAME(L) ↓				mAP (%) ↑
		$L = 1$	$L = 2$	$L = 3$	$L = 4$	
S-UNet	62.4 ± 55.4	66.9 ± 51.7	75.1 ± 50.6	95.3 ± 54.1	138.4 ± 75.2	66.8 ± 11.7
D-CSRNet	37.3 ± 41.0	45.7 ± 38.8	58.2 ± 38.5	77.6 ± 39.8	100.5 ± 45.0	27.7 ± 8.5
FRCNN	96.5 ± 128.0	103.8 ± 125.3	112.6 ± 121.9	133.9 ± 118.7	168.2 ± 123.2	57.9 ± 10.8

Finally, in Figure 2, we show some examples of predictions with very low absolute counting errors (suggesting good performance) but in which the average precision metric indicates erroneous predictions instead. Note that in the detection-based solution FRCNN, the disagreement between the two metrics is less pronounced, as this methodology is usually adopted to optimize AP. Thus, we suggest integrating the mean average precision, or at least a GAME-L metric with a high-enough L , when optimizing and evaluating novel cell counting solutions. We deem the additional evaluation protocol would help practitioners to better characterize the performance of developed solutions.

6 CONCLUSIONS

In this work, we consider the cell counting task in microscopy images, investigating the ability of three general counting methodologies not only in estimating the number of the biological structures but also in localizing them. Indeed, most state-of-the-art solutions tailored to cell counting are evaluated merely considering the difference between the predicted and

the actual number of the cells, skipping a further analysis focused on the quality of the provided estimations. We show that relying only on the counting metrics can lead to models producing incorrect cell localization. We performed experiments on three cell counting benchmarks, and we assessed that counting errors do not always agree with the localization performance. Thus, we suggest measuring and reporting also the mean average precision (or at least a grid average mean absolute error) whenever possible to help practitioners developing better models and to guide users to choose the model most tailored to their scenario.

ACKNOWLEDGEMENTS

This work was partially funded by: AI4Media - A European Excellence Centre for Media, Society and Democracy (EC, H2020 n. 951911), Extension (ESA, n. 4000132621/20/NL/AF), and AI-MAP (CNR4C program) - Tuscany POR FSE 2014-2020 (CUP B15J19001040004).

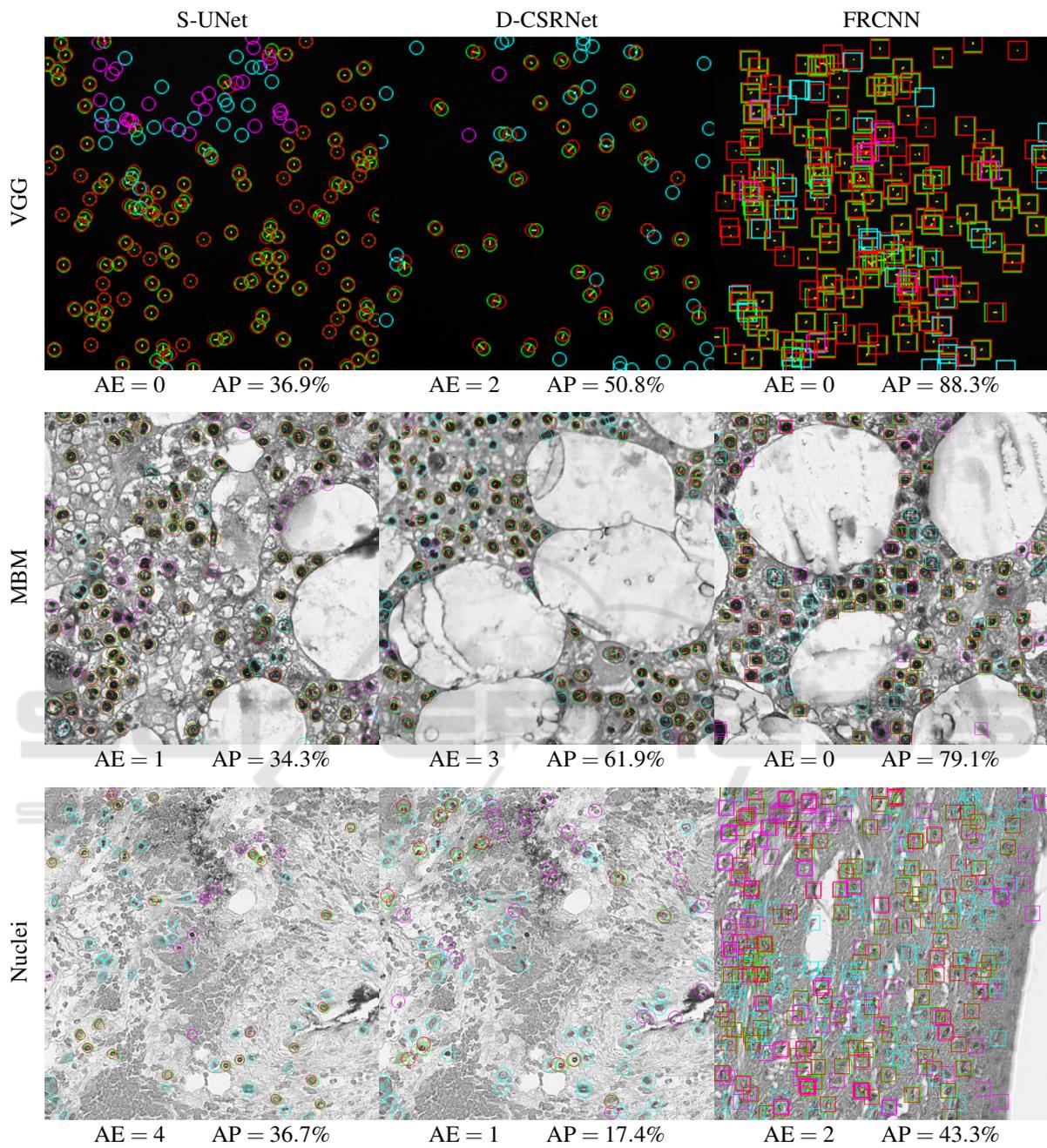


Figure 2: **Absolute Error (AE) can be misleading.** For each considered model (one per column), we show predictions obtaining a low AE, but also a low Average Precision (AP) due to high numbers of false positive and false negatives. The AP can discern cases where the MAE fails to capture poor model outputs. We indicate false positives in purple, false negatives in cyan, and true positives in green, with the corresponding ground-truth position drawn in red and connected via a thin yellow line. (Best viewed in electronic format.)

REFERENCES

- Aich, S. and Stavness, I. (2018). Improving object counting with heatmap regulation. *CoRR*, abs/1803.05494.
- Altman, S. A., Randers, L., and Rao, G. (1993). Comparison of trypan blue dye exclusion and fluorometric assays for mammalian cell viability determinations. *Biotechnology Progress*, 9(6):671–674.
- Amato, G., Bolettieri, P., Moroni, D., Carrara, F., Ciampi, L., Pieri, G., Gennaro, C., Leone, G. R., and Vairo, C. (2018). A wireless smart camera network for parking monitoring. In *2018 IEEE Globecom Workshops (GC Wkshps)*, pages 1–6. IEEE.
- Amato, G., Ciampi, L., Falchi, F., and Gennaro, C. (2019). Counting vehicles with deep learning in onboard UAV imagery. In *2019 IEEE Symposium on Computers and Communications (ISCC)*, pages 1–6. IEEE.
- Arteta, C., Lempitsky, V., Noble, J. A., and Zisserman, A. (2016a). Detecting overlapping instances in microscopy images using extremal region trees. *Medical Image Analysis*, 27:3–16.
- Arteta, C., Lempitsky, V. S., and Zisserman, A. (2016b). Counting in the wild. In *Computer Vision - ECCV 2016*, volume 9911, pages 483–498. Springer.
- Boominathan, L., Kruthiventi, S. S. S., and Babu, R. V. (2016). Crowdnet: A deep convolutional network for dense crowd counting. In *Proceedings of the 24th ACM international conference on Multimedia*, pages 640–644. ACM.
- Ciampi, L., Amato, G., Falchi, F., Gennaro, C., and Rabbitti, F. (2018). Counting vehicles with cameras. In Bergamaschi, S., Noia, T. D., and Maurino, A., editors, *Proceedings of the 26th Italian Symposium on Advanced Database Systems, Castellaneta Marina (Taranto), Italy, June 24-27, 2018*, volume 2161 of *CEUR Workshop Proceedings*. CEUR-WS.org.
- Ciampi, L., Gennaro, C., Carrara, F., Falchi, F., Vairo, C., and Amato, G. (2021a). Multi-camera vehicle counting using edge-ai. *CoRR*, abs/2106.02842.
- Ciampi, L., Santiago, C., Costeira, J., Gennaro, C., and Amato, G. (2021b). Domain adaptation for traffic density estimation. In *Proceedings of the 16th International Joint Conference on Computer Vision, Imaging and Computer Graphics Theory and Applications*, pages 185–195. SCITEPRESS - Science and Technology Publications.
- Ciampi, L., Santiago, C., Costeira, J. P., Gennaro, C., and Amato, G. (2020). Unsupervised vehicle counting via multiple camera domain adaptation. In Saffiotti, A., Serafini, L., and Lukowicz, P., editors, *Proceedings of the First International Workshop on New Foundations for Human-Centered AI (NeHuAI) co-located with 24th European Conference on Artificial Intelligence (ECAI 2020), Santiago de Compostella, Spain, September 4, 2020*, volume 2659 of *CEUR Workshop Proceedings*, pages 82–85. CEUR-WS.org.
- Cohen, J. P., Boucher, G., Glastonbury, C. A., Lo, H. Z., and Bengio, Y. (2017). Count-ception: Counting by fully convolutional redundant counting. In *2017 IEEE International Conference on Computer Vision Workshops (ICCVW)*, pages 18–26. IEEE.
- Falk, T., Mai, D., Bensch, R., Özgün Çiçek, Abdulkadir, A., Marrakchi, Y., Böhm, A., Deubner, J., Jäckel, Z., Seiwald, K., Dovzhenko, A., Tietz, O., Bosco, C. D., Walsh, S., Saltukoglu, D., Tay, T. L., Prinz, M., Palme, K., Simons, M., Diester, I., Brox, T., and Ronneberger, O. (2018). U-net: deep learning for cell counting, detection, and morphometry. *Nat. Methods*, 16(1):67–70.
- Fawcett, J. W., Oohashi, T., and Pizzorusso, T. (2019). The roles of perineuronal nets and the perinodal extracellular matrix in neuronal function. *Nat. Rev. Neurosci.*, 20(8):451–465.
- Guerrero-Gómez-Olmedo, R., Torre-Jiménez, B., López-Sastre, R., Maldonado-Bascón, S., and Oñoro-Rubio, D. (2015). Extremely overlapping vehicle counting. In *Pattern Recognition and Image Analysis*, pages 423–431. Springer International Publishing.
- Guo, Y., Krupa, O., Stein, J., Wu, G., and Krishnamurthy, A. (2021). SAU-net: A unified network for cell counting in 2d and 3d microscopy images. *IEEE/ACM Trans. Comput. Biol. Bioinform.*, pages 1–1.
- He, K., Gkioxari, G., Dollár, P., and Girshick, R. (2017). Mask r-cnn. In *Proceedings of the IEEE international conference on computer vision*, pages 2961–2969.
- He, S., Minn, K. T., Solnica-Krezel, L., Anastasio, M. A., and Li, H. (2021). Deeply-supervised density regression for automatic cell counting in microscopy images. *Medical Image Analysis*, 68:101892.
- Jiang, N. and Yu, F. (2020). A cell counting framework based on random forest and density map. *Appl. Sci.*, 10(23):8346.
- Johnston, G. (2010). Automated handheld instrument improves counting precision across multiple cell lines. *BioTechniques*, 48(4):325–327.
- Kainz, P., Urschler, M., Schuller, S., Wohlhart, P., and Lepetit, V. (2015). You should use regression to detect cells. In *Lecture Notes in Computer Science*, pages 276–283. Springer International Publishing.
- Kotoura, Y., Yamamuro, T., Shikata, J., Kakutani, Y., Kit-sugi, T., and Tanaka, H. (1985). A method for toxicological evaluation of biomaterials based on colony formation of v79 cells. *Archives of Orthopaedic and Traumatic Surgery*, 104(1):15–19.
- Laradji, I. H., Rostamzadeh, N., Pinheiro, P. O., Vazquez, D., and Schmidt, M. (2018). Where are the blobs: Counting by localization with point supervision. In *Computer Vision – ECCV 2018*, volume 11206, pages 560–576. Springer International Publishing.
- Lempitsky, V. S. and Zisserman, A. (2010). Learning to count objects in images. In *Advances in Neural Information Processing Systems 23: 24th Annual Conference on Neural Information Processing Systems 2010*, pages 1324–1332. Curran Associates, Inc.
- Li, Y., Zhang, X., and Chen, D. (2018). CSRNet: Dilated convolutional neural networks for understanding the highly congested scenes. In *2018 IEEE/CVF Conference on Computer Vision and Pattern Recognition*, pages 1091–1100. IEEE.
- Lu, E., Xie, W., and Zisserman, A. (2019). Class-agnostic

- counting. In *Computer Vision – ACCV 2018*, pages 669–684. Springer International Publishing.
- Lugagne, J.-B., Lin, H., and Dunlop, M. J. (2019). DeLTA: Automated cell segmentation, tracking, and lineage reconstruction using deep learning.
- Marsden, M., McGuinness, K., Little, S., Keogh, C. E., and O'Connor, N. E. (2018). People, penguins and petri dishes: Adapting object counting models to new visual domains and object types without forgetting. In *2018 IEEE/CVF Conference on Computer Vision and Pattern Recognition*. IEEE.
- Oñoro-Rubio, D. and López-Sastre, R. J. (2016). Towards perspective-free object counting with deep learning. In *Computer Vision – ECCV 2016*, volume 9911, pages 615–629. Springer International Publishing.
- Paulauskaite-Taraseviciene, A., Sutiene, K., Valotka, J., Raudonis, V., and Iesmantas, T. (2019). Deep learning-based detection of overlapping cells. In *Proceedings of the 2019 3rd International Conference on Advances in Artificial Intelligence*, pages 217–220. ACM.
- Ren, S., He, K., Girshick, R., and Sun, J. (2017). Faster r-CNN: Towards real-time object detection with region proposal networks. *IEEE Trans. Pattern Anal. Mach. Intell.*, 39(6):1137–1149.
- Ronneberger, O., Fischer, P., and Brox, T. (2015). U-net: Convolutional networks for biomedical image segmentation. In *Medical Image Computing and Computer-Assisted Intervention - MICCAI 2015*, volume 9351 of *Lecture Notes in Computer Science*, pages 234–241. Springer.
- Segui, S., Pujol, O., and Vitria, J. (2015). Learning to count with deep object features. In *2015 IEEE Conference on Computer Vision and Pattern Recognition Workshops (CVPRW)*. IEEE.
- Simonyan, K. and Zisserman, A. (2015). Very deep convolutional networks for large-scale image recognition. In *3rd International Conference on Learning Representations, ICLR 2015*.
- Sirinukunwattana, K., Raza, S. E. A., Tsang, Y.-W., Snead, D. R. J., Cree, I. A., and Rajpoot, N. M. (2016). Locality sensitive deep learning for detection and classification of nuclei in routine colon cancer histology images. *IEEE Transactions on Medical Imaging*, 35(5):1196–1206.
- Szegedy, C., Liu, W., Jia, Y., Sermanet, P., Reed, S., Anguelov, D., Erhan, D., Vanhoucke, V., and Rabinovich, A. (2015). Going deeper with convolutions. In *2015 IEEE Conference on Computer Vision and Pattern Recognition (CVPR)*, pages 1–9. IEEE.
- Xie, W., Noble, J. A., and Zisserman, A. (2016). Microscopy cell counting and detection with fully convolutional regression networks. *Comput. methods Biomech. Biomed. Eng. Imaging Vis.*, 6(3):283–292.
- Xie, Y., Xing, F., Shi, X., Kong, X., Su, H., and Yang, L. (2018). Efficient and robust cell detection: A structured regression approach. *Medical Image Analysis*, 44:245–254.
- Yu, F. and Koltun, V. (2016). Multi-scale context aggregation by dilated convolutions. In *4th International Conference on Learning Representations, ICLR 2016*.
- Zhang, F., Qian, X., Si, H., Xu, G., Han, R., and Ni, Y. (2015). Significantly improved solvent tolerance of *escherichia coli* by global transcription machinery engineering. *Microbial Cell Factories*, 14(1).