# Transfer Learning for the Recognition of Immunogold Particles in TEM imaging

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**Abstract** We present a Transfer Learning (TL) framework based on Stacked Denoising Autoencoder (SDA) for the recognition of immunogold particles. These particles are part of a high-resolution method for the selective localization of biological molecules at the subcellular level only visible through Transmission Electron Microscopy (TEM). Four new datasets were acquired encompassing several thousands of immunogold particles. Due to the particles size (for a particular dataset a particle has a radius of 4 pixels in an image of size $4008 \times 2670$) the annotation of these datasets is extremely time taking. Thereby, we apply a TL approach by reusing the learning model that can be used on other datasets containing particles of different (or similar) sizes. In our experimental study we verified that our TL framework outperformed the baseline (not involving TL) approach by more than 20% of accuracy on the recognition of immunogold particles.

## 1 Introduction

Common Machine Learning create new classifiers whenever the respective probability distributions of inputs and outputs change, even though they may relate to similar problems. The *reuse* of a classifier designed for a given (*source*) problem on another (*target*) problem, presenting some similarities with the original one, with only minor operations of parameter tuning, is the scope of Transfer Learning (TL).

This paper proposes a TL framework for the recognition of immunogold particles on datasets with different magnifications. The structure of this framework is as follows: (i) a Stacked Denoising Autoencoder (SDA) model is built to recognize particles of a specific size; (ii) in order to ease the burden of image annotation and aiming for an effective particle recognition a robust SDA obtained through TL is developed. To the best of our knowledge this work is the first TL

framework of its kind for immunogold particle recognition. Furthermore, we also make publicly available a new dataset that can be used as benchmark on future works.

Immunogold electron microscopy is a high-resolution method for the selective localization of biological molecules at the subcellular level. Antibodies coupled to particles of colloidal gold, which are visible through Transmission Electron Microscopy (TEM), can reveal the localization and distribution of the biological molecules of interest. In this particular work, this technique was used to determine the composition of cell wall uneven thickenings that ultimately differentiate into reticulate and flange ingrowths of maize (*Zea mays* L.) endosperm transfer cells [15]. These cells are essential for assimilate flow into the endosperm, thus having a significant impact on kernel yield.

Immunogold particle counting is a time-consuming task where a single image containing almost a thousand particles can take several hours to annotate [19] (Figure 1). Moreover, recent technological improvements in microscopy have made easier and faster to acquire large volumes of data of biological samples at very high, Electron Microscopy (EM)-level, magnifications. To add, images are usually recorded with different magnifications for a better analysis of the biological tissues. Conducting this analysis on a daily basis is not only a tiresome task but is also very time consuming. An automatic recognition tool can reduce the time consumed in such analysis and improve its accuracy (by removing the bias of a manual annotation) thus being of paramount importance. Ultimately, through TL we can relax the necessity of obtaining large amounts of labeled data for new problems [2, 5] (such as new magnifications); and, less computational effort by providing new learning models that can be applied with good performance results in different problems and far less computational effort (see e.g., [5, 10, 11]).

This paper is structured in the following way: we start by briefly describing previous works on the recognition of cellular structures aided by automatic systems (Section 2). In Section 3 we present our TL framework for the recognition of immunogold particles. In Section 4 we present the results and the conclusions are drawn in Section 5.

## 2 Previous Work

Fisker *et al.* in [8] explores the possibility to automatically estimate particle sizes in immuno-microscopy imaging. Their approach is based on deformable models that can be fitted to the prior known shape of the particles. With the same goal as Fisker, a different approach was presented by Mallic *et al.* in [13] by using cascade of classifiers. Inspired in the conventional face detection challenges where recognition with ensemble of weak classifiers have proven to be effective, this straightforward strategy may be computationally complex due to the amount of immunogold particles that may occur in electron microscopy images. In [19] an insight review is presented by motivating the advantages of Computer Vision (CV) for the processing of images generated by electron microscopies. Finally,

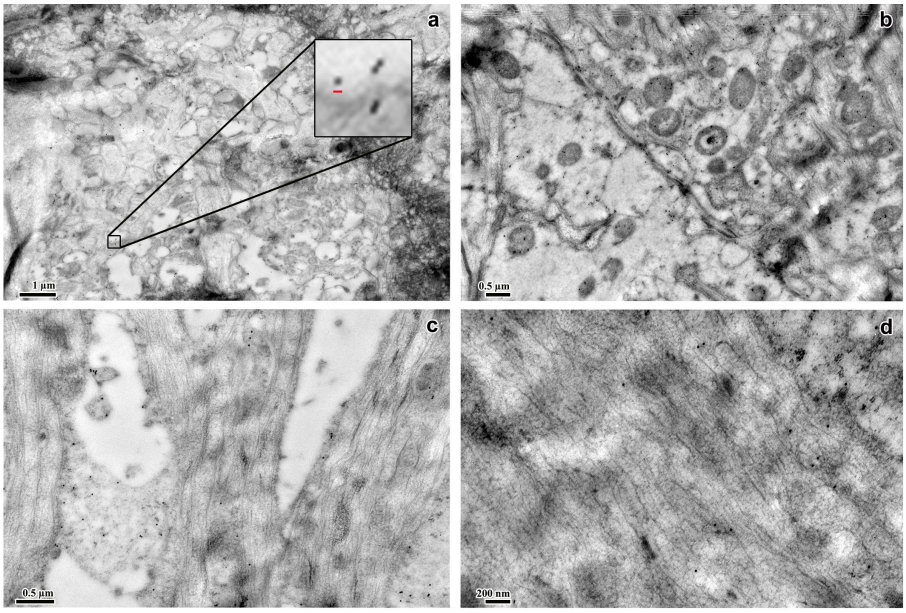


Figure 1: Representative images of our datasets illustrating different structures that can interfere in the recognition of the immunogold particles due to: cellular overlapping, tissues and background noise. Each image has $4008 \times 2670$ pixels of dimension with particles *diameter* ranging from 8 to 20 pixels. **(a)** Example of a sample with a magnification of 15000 ($1\mu m$, particles with a diameter of 8 pixels—red line); **(b)** magnification of 20000 ($0.5\mu m$, 12 pixels diameter particles); **(c)** magnification of 30000 ($0.5\mu m$, 15 pixels diameter particles); and, **(d)** magnification of 50000 ($200nm$, 20 pixels diameter particles).

in [23, 24] a Difference of Gaussian (DoG) and Laplacian of Gaussian (LoG) filters are applied to aid the detection of particles (e.g., organelles) on cryo-electron microscopy images. On these papers, DoG or LoG were used as a first step to detect more complex biological structures and were not tailored neither evaluated on immunogold particles. For the *detection* of biological structures, there is the publicly available Spot Detector (SD) [17] algorithm that is included in the well-known Icy bioimaging software [7]. Authors state that SD enables the detection of spots that can be organelles or other biological similar structures in noisy 2D or 3D images [17].

A major difference is that in the aforementioned proposals all particle structures were shallow, irregular in shape and intensity. Our work will be focused on the recognition of immunogold particles with regular spherical shape, thus avoiding the adoption of a highly parameterized formalism for its recognition. Furthermore, the creation of different models, each one developed for a specific

problem, can be cumbersome due to the time that is needed to label the data. For this purpose TL is presented as an appealing alternative.

TL has been around since the 80's with considerable advancements since then (see e.g., [9, 14, 18, 21] and references therein). With the recent re-interest on Neural Networks (NNs) and the availability of more computational power along with new and faster algorithms, NNs with deep architectures started to emerge to tackle TL. In [12] (and work referenced therein) the authors have widened the research line on TL by addressing the following questions: How can one tailor Deep Neural Networks (DNNs) for TL? How does TL perform by reusing layers and using different types of data? For the application of Microscopy Imaging a TL categorization was presented in [4] for the recognition of mitochondria. With the exception of [16] where an adaboost and TL framework is proposed for the detection of cells with different features (e.g., shape, size), there are very few works that address the possibility of TL on biomedical data, specifically for the recognition of immunogold particles.

Current approaches are not tailored for immunogold particle detection and recognition and thus are prone to error by erroneously estimating a microstructure organelle or an artifact as immunogold particle due to the criteria of the filter responses: circular shape and radius dimension. To this end a robust mechanism is required to handle possible false detections that can occur in this kind of images. In the following Sections we will present a SDA-based TL mechanism as an effective approach for immunogold particle recognition.

## 3 A TL framework for the Recognition of Immunogold Particles using SDAs

Our proposal comprises the following two steps:

- a SDA is first trained to distinguish immunogold particles from cluttered background;
- the (learning) model is then reused onto another dataset (with particles of a different size).

In the following sections we will thoroughly describe the methods (for a self-contained reading) and the framework here proposed.

### 3.1 SDAs for the Recognition of Immunogold Particles

An autoencoder is a simple NN with one hidden layer designed to reconstruct its own input, having, for that reason, an equal number of input and output neurons. The reconstruction accuracy is obtained by minimizing the average reconstruction error between the original and the reconstructed instances. Hence, these methods are governed by the objective of capturing relevant information of the underlying distribution of the samples [20]. The encoding and decoding feature sets (input-hidden and hidden-output weights, respectively) may optionally be constrained as transpose of each other, in which case the autoencoder is

said to have tied weights. For a variant of the autoencoders, Denoising Autoencoder (DAE), input data is corrupted to train a model in order to rebuild the original data. Such is achieved by means of the minimization of some reconstruction loss and allows the DAEs to avoid a direct copy of the data [22]. Stacking autoencoders, gives the model the advantage of hierarchical features with low-level features represented at lower layers and higher-level features represented at upper layers [6, 22, Section 3]. The unsupervised training of the SDAs is usually referred to as pre-training. We then add to the top of the network a logistic layer and the entire network is "fine-tuned" in order to minimize some classification loss function [1, 6].

SDAs robustness makes it a very promising learning tool for the recognition of the circular shaped immunogold particles. A representative sample of the images that were used to train the SDA is depicted in Figure 2. Given the

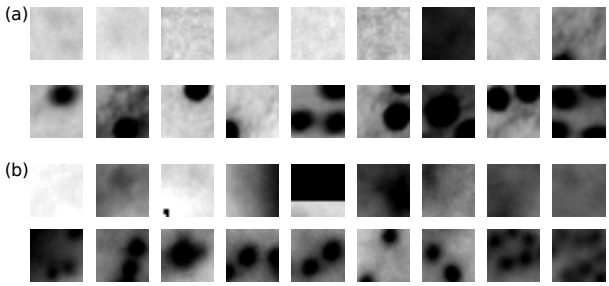


Figure 2: different types of patches in the dataset with magnification of 50000 (a): background and artifact patches (two top rows); and, patches containing at least one immunogold particle (two bottom rows); and, (b) analogous for the patches in the dataset with magnification of 15000.

similarity of the immunogold particles, it is expected that a SDA can capture relevant features from these samples and easily discriminate from the remaining artifacts or cellular structures. A far more complex scenario occurs when multiple immunogold particles are comprised in the same patch (see Figure 2), especially in low magnifications such as the magnification of 15000. In this case the SDA has to be able to deal with a variable number of particles in the same image. Besides this, it also has to be robust to the different particle sizes.

Although it may seen unsual to capture images in different magnifications, the automatic analysis in lower magnifications (magnification of 15000) helps a direct identification of regions of interest and its direct quantification as opposed to when using higher magnifications. By directly exploring images obtained at lower magnifications we can attain reduced experimentation costs, image acquisition and labelling times. However, the performance of an automatic learning model for these images is prone to misclassify a great number of images due to the noise and artifacts present in the dataset. For this, we can build a learning model using a dataset from higher magnifications, such as magnification of 50000

(containing immunogold particles well defined), and transfer it to a magnification of 15000.

Therefore, a TL setting for the recognition of immunogold particles without requiring the learning of new models from scratch (e.g., by initializing the layer weights at random) is important.

### 3.2 TL: reusability of SDA models

TL aims to transfer models acquired in one problem, the source problem, onto another problem, the target problem, dispensing the bottom-up construction of the target model. TL algorithms embedded in DNNs have already shown interesting features such as better generalization capabilities and reduced training times as shown in other works [10] (and references therein). In here we extend these studies of model reusability to the recognition of immunogold particles in images of maize endosperm transfer cells. A dataset for a given magnification is identified as the source problem and the (source) learning model will be built upon this data. TL is then applied by reusing the source model on the target problem (in this case, a dataset with a different magnification). Putting it simply, layer weights for the target model are initialized with the values of the source model. Learning (of the target problem) can be conducted in several ways: (a) by fixing the layer weights of the network or let them readjust through the minimization of the reconstruction error (also known as pre-training); (b) by fixing or letting the network relearn and thus letting it readjust the decision function (fine-tune); or (c) a mixture of both [2, 3, 6, 10, 11] (and references therein).

For the purpose of this work, we have reused the source model on the target problem by letting it readjust the decision function to the target problem. In our experimental work we have analyzed the impact of the reusability of the different hidden layers. In fact, and for the immunogold particle recognition, one may argue that the major changes will occur at a feature level thanks to the changes of the magnification of the immunogold particles. Looking carefully at Figure 1 we can see that this seems to be the case. Therefore, the recognition performance of the immunogold particles through TL would have significant impacts by relearning only the first layers of the network. The analysis of the effects of relearning specific parts of the network architecture will be addressed in more detail in Section 4.

## 4 Methodology and Results Discussion

### 4.1 Dataset

All images were acquired using a Transmission Electron Microscope JEOL JEM 1400 with a GATAN Orius SC10000A2 CCD and were recorded in four different magnifications: 15, 20, 30 and 50 thousand times from different biological samples. See Figure 1 for a brief illustration of this dataset. For each magnification we recorded 25 digital images. Each image contains 500 to 200 particles annotated by a trained expert (localization on the image of an immunogold particle).

### 4.2 Experimental Study

**Dataset for SDA Models Construction:** For a given image we extracted *all* immunogold particles and the same amount of background, cellular structures or artifacts patches with $20 \times 20$ pixels (the latter at random positions). A patch could contain more than one particle or portions of several other particles (see Figure 2). Finally, patches were labeled as containing at least one immunogold particle if the Euclidean distance between the patch central position (on the image) and the annotation position was below 10 pixels (half of the size of the patch width). Moreover, we ensure that there is a one-to-one mapping between the patch location and the ground-truth.[8] This procedure thus results on a dataset (for a given magnification) containing several thousand patches of immunogold particles and background, cellular structures or artifacts.

**Training Procedure:** The experimental procedure for the construction of the baseline models was conducted as follows. For a given resolution we randomly split the data into training and test data: 60% to train our SDA (60 images in total, 15 samples per resolution) and the remaining for testing purposes. Pixel intensity values of all patches were normalized to be within $[0, 1]$. To find the best SDA model parameterization we have performed a grid search on the pre-training learning rate (0.01 and 0.001) and fine-tuning learning rate (0.1 and 0.01) by carrying out a three-fold cross-validation in the training set. The input layer had 400 neurons (for handling the patches size of $20 \times 20$). The number of hidden layers was fixed to 3 and the number of neurons per layer was fixed to 500. The corruption level was set to 0.1 across all hidden layers. Regarding TL, we proceeded similarly in order to find the best fine-tune learning rate. Due to the different amount of samples, we also performed a cross-validation over the batch-size. Finally, to assess the variability of our methods' performance the experiment was repeated 20 times by randomly shuffling the data.

**TL evaluation:** The procedure described above, conducted for a single magnification, allows us to obtain our baseline (without TL) model. For this particular study, such was conducted for the dataset with magnification of 15000 (target problem). Then, we have proceeded with the TL approach. A learning model is trained on the dataset with magnification of 50000 (source problem). Images of this dataset were resized to half (keeping the aspect ratio) of our source problem, but maintaining the size of the patches ($20 \times 20$). This led to immunogold particles with a radius around 7 pixels (recall that the radius size of immunogold particles on the dataset with magnification of 15000 was 4 pixels). The model obtained in the source problem was then reused for learning the dataset with a magnification of 15000 (target problem).

In our framework, TL can be conducted by reusing the first layer of the model and by fine-tuning only the remaining layers to the target problem (coded [011]); another possibility is reusing the first and second layers and fine-tuning

[8] A given manual position will not result on more than one patch.

the last layer ([001]). By reusing the full network ([000]) the model would not suffer changes, and thus would have not been fine-tuned to the target problem. Results are presented in Table 1.

| Method | Source | Target | Reusability and Fine-Tuning (TL) Setting | Accuracy (± std. dev) |
|---|---|---|---|---|
| Baseline | - | 15000 | - | $0.65 \pm 0.17$ |
| TL | 50000 | 15000 | [011] | $0.88 \pm 0.01$ |
| TL | 50000 | 15000 | [001] | $0.86 \pm 0.02$ |
| TL | 50000 | 15000 | [110] | $0.90 \pm 0.01$ |
| TL | 50000 | 15000 | [100] | $0.88 \pm 0.01$ |
| TL | 50000 | 15000 | [111] | $\mathbf{0.91 \pm 0.01}$ |

Table 1: Results of the application of TL to the recognition of immunogold particles. The baseline model was trained in a standard ML way on the dataset with magnification of 15000 (target problem). A model trained for the dataset with magnification of 50000 (source problem) was obtained and it was reused on the target problem. Overall, all TL approaches achieved an improvement of more than 20%. Each layer-wise TL strategy is illustrated in the column TL setting (see main text).

**Discussion:** Considering the results shown in Table 1 we see that by applying any of the TL settings we can indeed improve the baseline results (65%). First, we speculate that the reason for the low accuracy rate of the baseline is concerned with the high variability location of the particles. Having smaller sizes (radius of 4 pixels), and given the unconstrained approach of the dataset construction (see Section 3.1), the network will be biased to underperform on the immunogold recognition. Second, the dataset with magnification of 15000 contains a significant number of clusters of immunogold particles increasing the difficulty of processing patches from this dataset. Moreover, this dataset is also comprised of a significant amount of artifacts (see Figure 1). Therefore, by learning a more well defined (source) problem (dataset with magnification of 50000), though still acquired through an unconstrained approach, a better starting point for learning the target problem is provided. Transferring the model and letting all layer weights be fine-tuned ([111]) to the target problem achieved the best result (91%). Another result from our experimental study is concerned with the relevance of the feature representation that the network has captured. From Table 1 we can infer that the first layers are the most relevant. In fact, letting the first two layers of the network be re-learnt and fixing only the last layer (reusability setting [110]) achieved a higher performance (90%) in comparison with only re-learning the last layer ([001] with an accuracy of 86%).

## 5 Conclusions

Current approaches for the quantification and analysis of histological datasets of transfer cells are essentially manual processes. This procedure is highly time-consuming, prone to error and is suitable for an automated processing by a computer. For the immunogold particles quantification there are no algorithms that claim to be able to automate tasks such as the immunogold labelling. Moreover, recent technological improvements in microscopy have made easier and faster the acquisition of large volumes of data of biological samples at very high, Electron Microscopy (EM)-level, magnifications. Its automatic recognition is therefore of paramount importance.

In this work we have presented a Transfer Learning (TL) framework based on Stacked Denoising Autoencoder (SDA) for automatic classification of immunogold particles with different magnifications. Our results have shown the robustness of the SDA given the amount of noise present in TEM images from Maize samples (see Figure 2). We have also illustrated the robustness of the possible TL settings for the reusability of the networks by attaining accuracy improvements of more than 20% in comparison to the baseline approach. We were also able to show the relevance of the features that were learnt in the first layer so that the network could be tailored to the new (target) problem.

For future work we plan to assess the reusability of the network on other datasets with immunogold particles with more drastic differences of sizes. This would allow us to assess the robustness of the SDA with respect to the invariance of particle size. This will be accomplished by exploring the remaining magnifications. In the future, we will also consider other Deep Neural Network (DNN) architectures.

## Acknowledgement

We would like to acknowledge to Fundação para a Ciência e a Tecnologia (FCT) for funding this research through project SFRH/BD/80508/2011, PDTC/EIA-EIA/119004/2010 and PEst-C/SAU/LA0002/2013. Sara Rocha was supported by Grant BIIC M3.1.6/F/038/2009 from Direcção Regional de Ciência e Tecnologia and by Grant SFRH/BD/8122/2002 from FCT. We thank Rui Fernandes from HEMS department for aiding us with the technical knowledge for conducting this work.

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