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A label free quantification of gold nanoparticle at the single cell level using multi-column convolutional neural network (MC-CNN)

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Supplementary document

1. **Spectral analysis using nanoparticle plasmon coupling and higher order image correlation spectroscopy:** We investigated the plasmon coupling effect of different sized nanospheres using FDTD simulation. From the FDTD simulation it was observed that, for 80 nm diameter Au monomers and dimers (1 nm separation) peak and intensity were 558 nm and 1.65571×10−14 m2, (1 nm separation) 7.85 nm and 8.43×10−14 m2 and 8.77 nm respectively. Integrating the total spectrum, we extracted the dimer to monomer ratio for 80 nm diameter AuNSs. The ratio of dimer to monomer varies from 2 ~ 4, similarly the ratio of trimer to monomer varies from 4 ~ 9 for different separations.

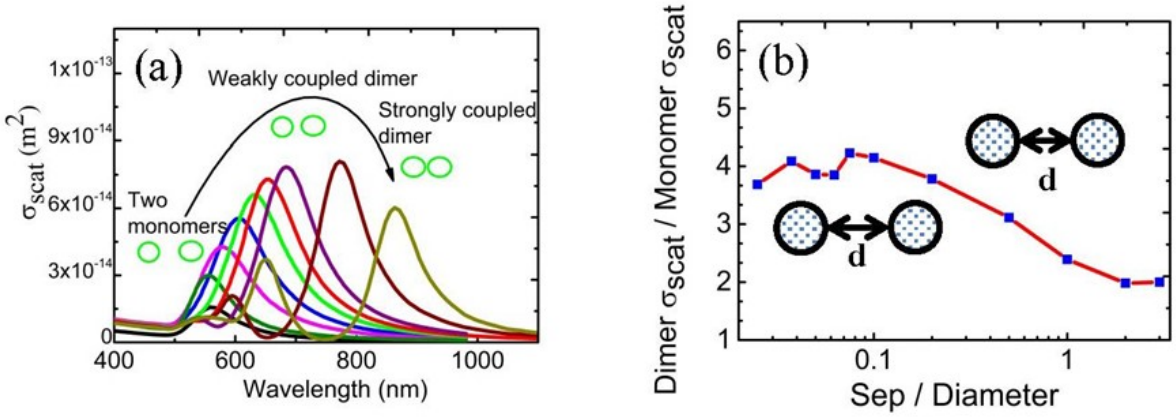

Fig. S1. Finite difference time domain simulations and quantum yield of 80 nm diameter gold nanosphere (AuNSs), (a) scattering cross - sections of 80 nm diameter AuNS at different separation and (b) quantum yield with respect to separation/diameter. The total integrated scattering cross section spectrum in the visible to near-infrared region (500 nm ~ 900 nm) plotted with respect to the separation distance for 80 nm diameter particles. The total strength of the dimer cross section is between 2 to 4 times that of the monomer which corresponds to the increase in scattering intensity with volume squared in the electrostatic limit.

Afterwards we plugged in the cluster to monomer ratio in the spatial high order autocorrelation function of order m and n, $G_{m,n}(\xi, \eta)$, which is defined as

$$G_{m,n}(\xi,\eta) = \frac{<\delta i(x,y)^m><\delta i(x+\zeta, y+\eta)^n> - <\delta i>^m <\delta i>^n}{<i>^{m+n}} \quad (1)$$

where $(m, n) \epsilon N$ are positive integers that determine the order of the image. Considering many different species of aggregate are present in the system, peak values of the high order mode can be expressed by the following equation suggested by Palmer and co – workers.[1]

$$G_{1,1}(0) = B_2$$
$$G_{1,2}(0) = 4B_3 / 3$$
$$G_{2,2}(0) = 2B_4 + 2B^2{}_2$$
$$G_{1,3}(0) = 2B_4 + 3B^2{}_2$$
$$G_{2,3}(0) = 16B_5 / 5 + 12B_2 B_3$$
$$G_{3,3}(0) = 16B_6 / 3 + 30B_2 B_4 + 15B^3{}_2 + 16B^2{}_3 \quad (2)$$

$$B_k = \frac{\sum_{i=1}^{R} \alpha_i^k \langle N_i \rangle}{\left[\sum_{i=1}^{R} \alpha_i \langle N_i \rangle\right]^k},$$

Here, α_i is the emitting quantum yield ratio of the aggregate (Q_i) to that of the monomer (Q_1), $\alpha_i = \frac{Q_i}{Q_1}$ and $< N_i >$ is the concentration of the i th oligomer.

If there are only two populations of oligomers, i.e. monomer and dimer, then only the first three higher order normalized moments need to be considered.

$$B_2 = \frac{\langle N_1 \rangle + \alpha_2^2 \langle N_2 \rangle}{\left[\langle N_1 \rangle + \alpha_2 \langle N_2 \rangle\right]^2}$$

$$B_3 = \frac{\langle N_1 \rangle + \alpha_2^3 \langle N_2 \rangle}{\left[\langle N_1 \rangle + \alpha_2 \langle N_2 \rangle\right]^3}$$

$$B_4 = \frac{\langle N_1 \rangle + \alpha_2^4 \langle N_2 \rangle}{\left[\langle N_1 \rangle + \alpha_2 \langle N_2 \rangle\right]^4}$$

The real solutions of the above equations represent the population densities of two species $<N_1>$ monomer concentration, $<N_2>$ dimer concentration, and the dimer quantum yield ratio α_2. By determining the six high order moments of the above equations (i.e., $B_2 \sim B_7$), we can solve the parameters for three emitting species. If more species are included in one image, simultaneous equations of higher order will have to be

solved to extract information about the samples. For this analysis we considered dimer as higher order oligomer[2].

2. Performance Metrics

$$APE = \frac{|Predicted - Actual|}{Actual} * 100$$

The Absolute Percentage Error (APE) is the percentage error between a single predicted value and actual value. As we are concerned with comparing two different values for the count of particle uptake, this is the most suitable metric.

3. Training and Loss-Accuracy Curves

The following section gives the Loss-Accuracy curves for the Training set and Validation set during the process of training each of the model architectures tested.

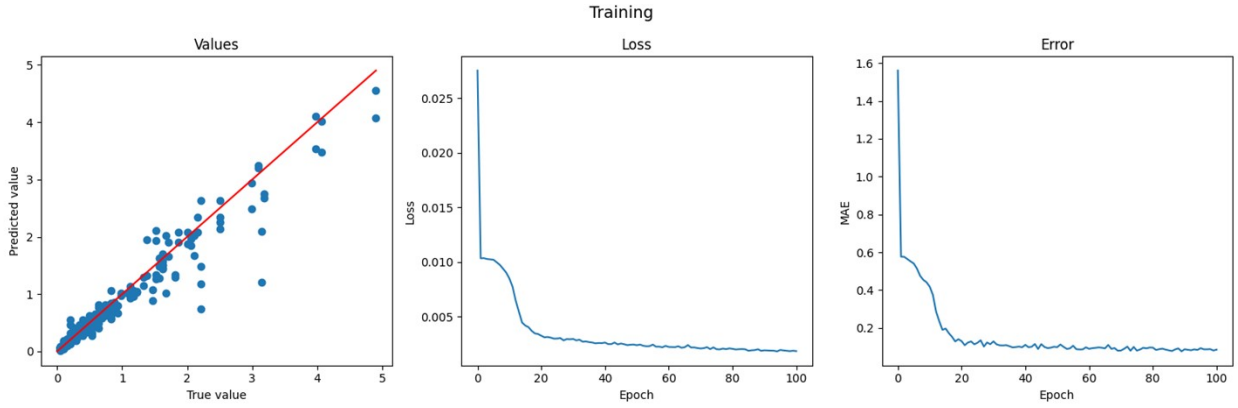

Fig. S2. Loss-Accuracy Curves for training the FCRN-A model.

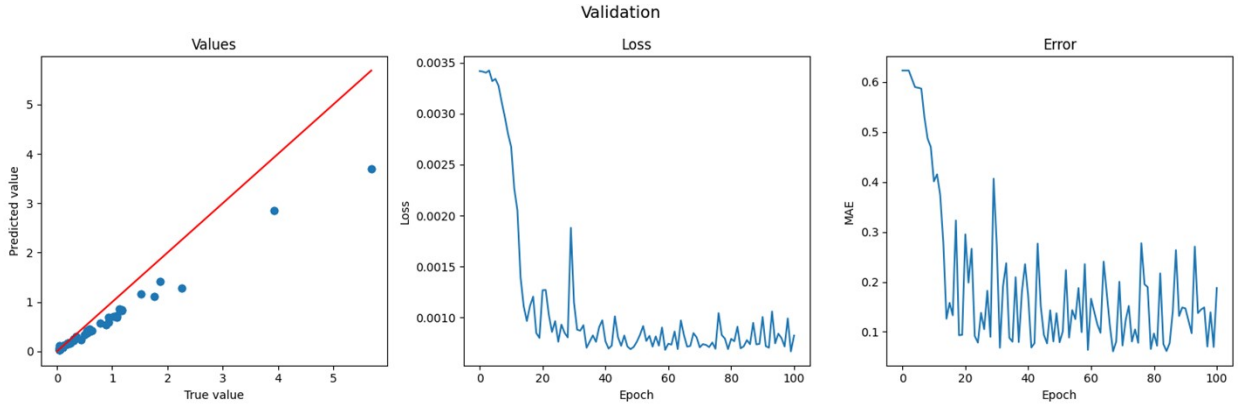

Fig. S3. Loss-Accuracy Curves for validating the FCRN-A model.

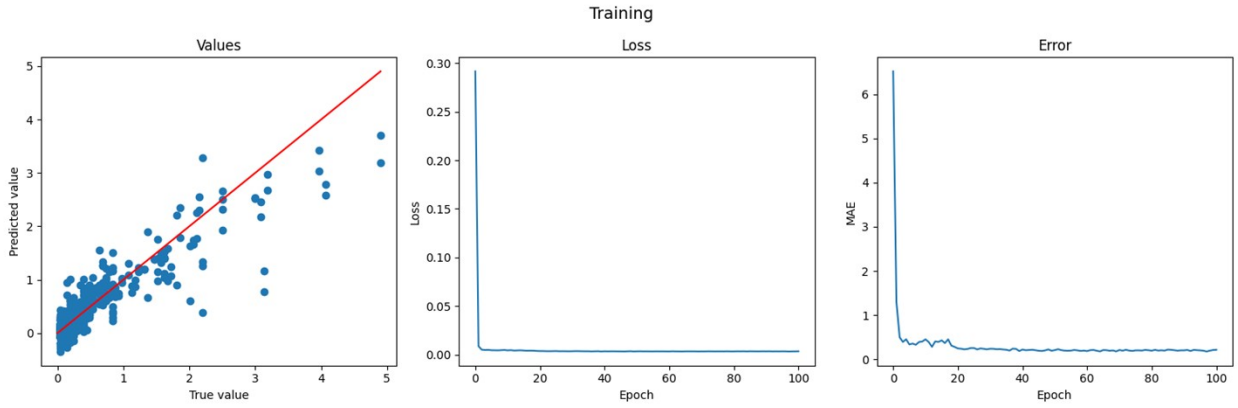

Fig. S4. Loss-Accuracy Curves for training the UNet model.

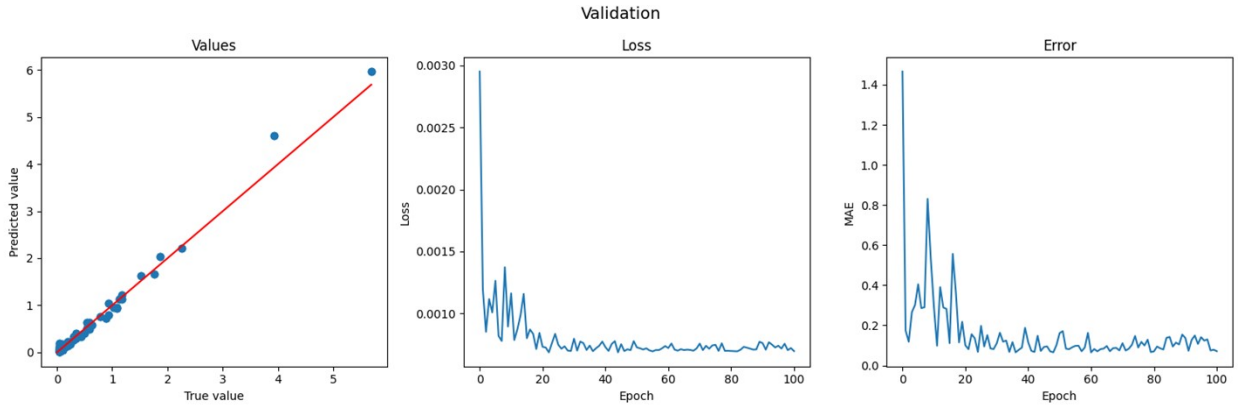

Fig. S5. Loss-Accuracy Curves for validating the UNet model.

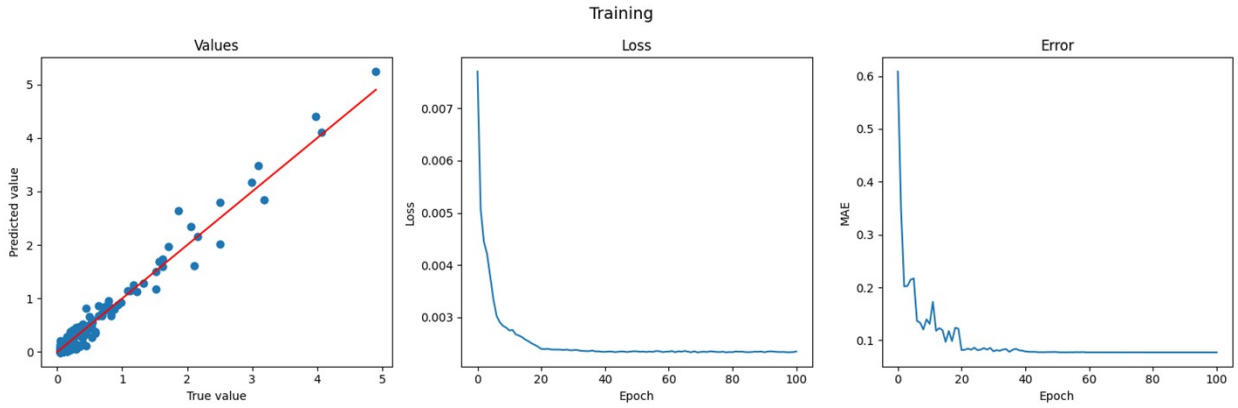

Fig. S6. Loss-Accuracy Curves for training the MC-CNN model.

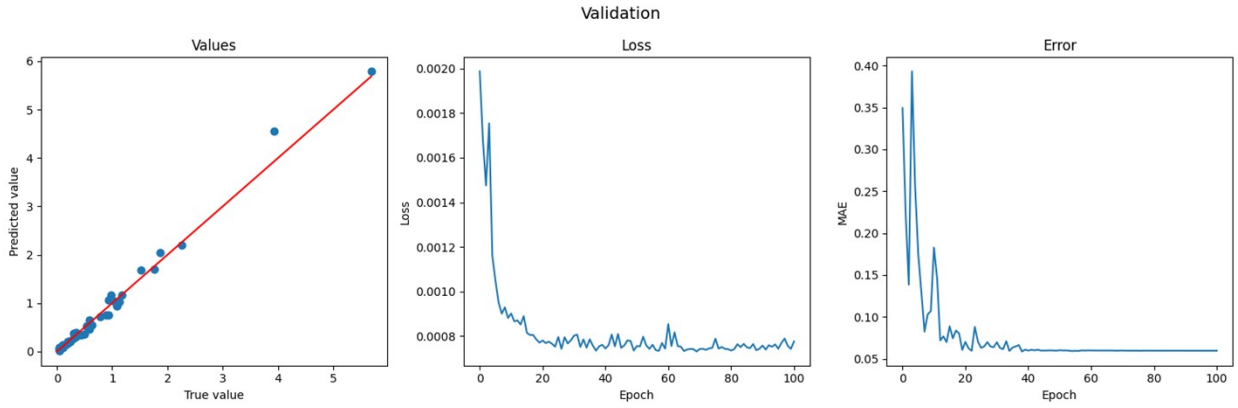

Fig. S7. Loss-Accuracy Curves for validating the MC-CNN model.

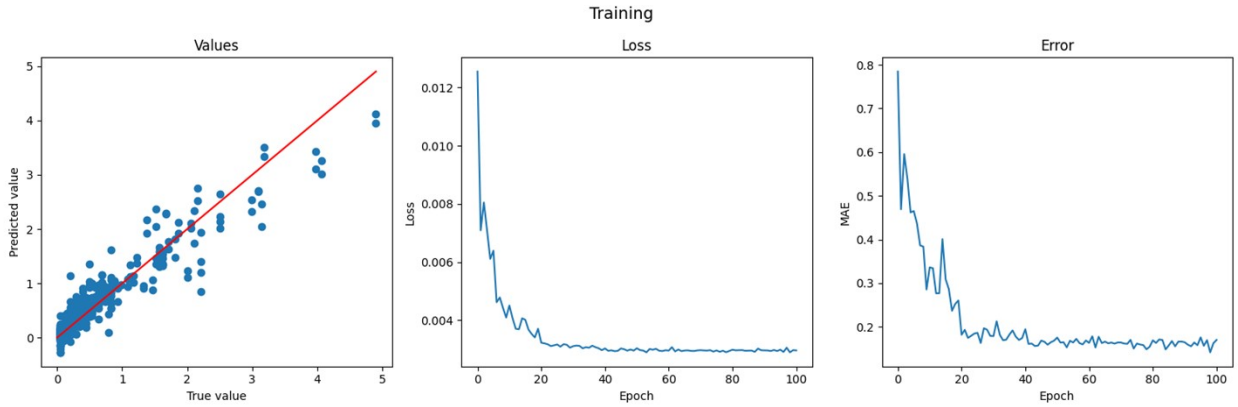

Fig. S8. Loss-Accuracy Curves for training the Trans-UNet model.

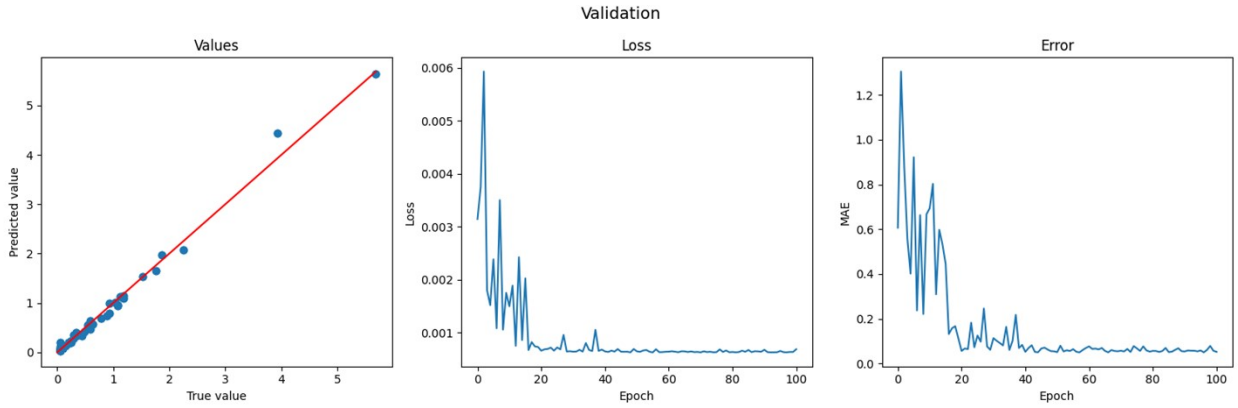

Fig. S9. Loss-Accuracy Curves for validating the Trans-UNet model.

4. Incubation Images

The following images were captured at different incubation periods using the methods outlined in Section 3.1 of the paper. These images comprised our Test set.

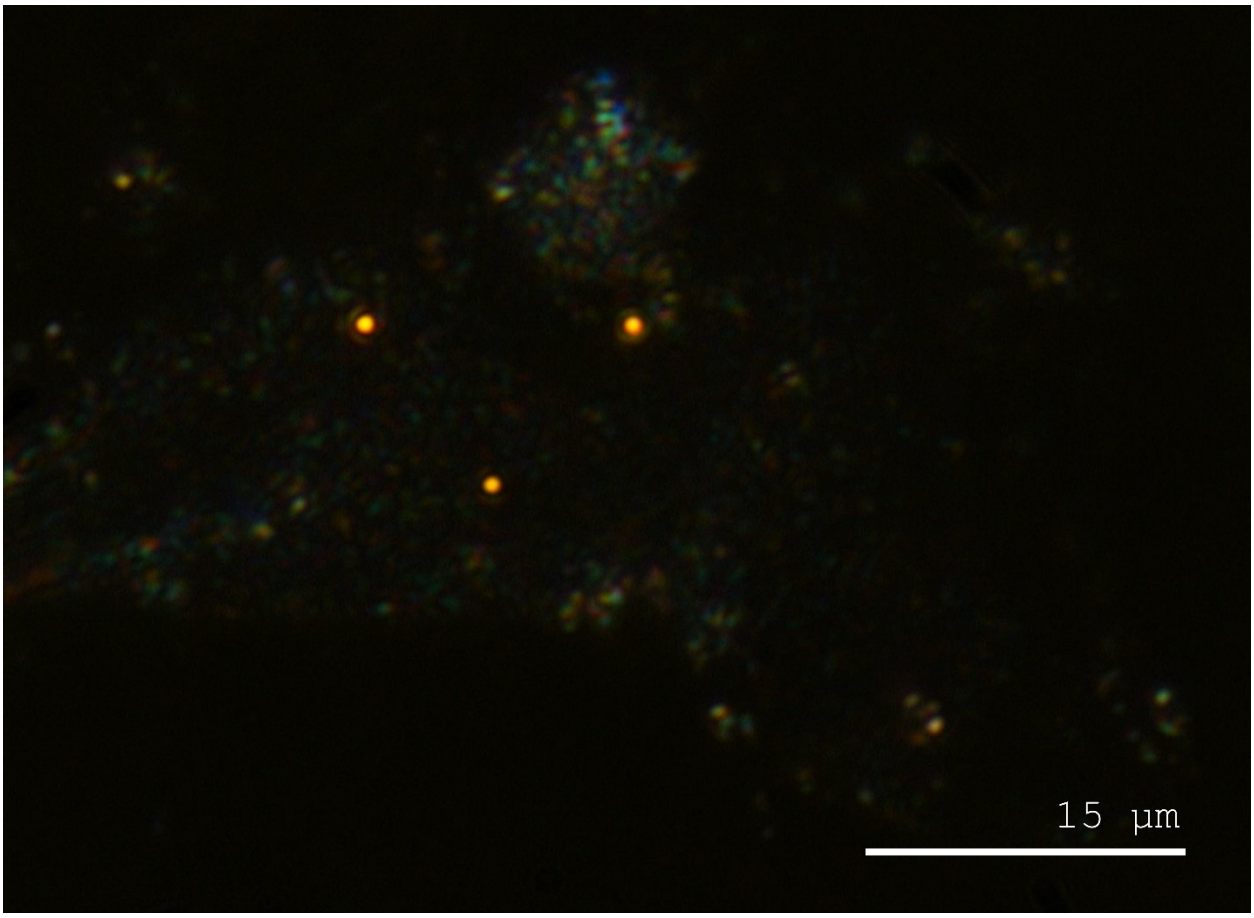

Fig. S10. Cell Imaged after 6 hours of incubation.

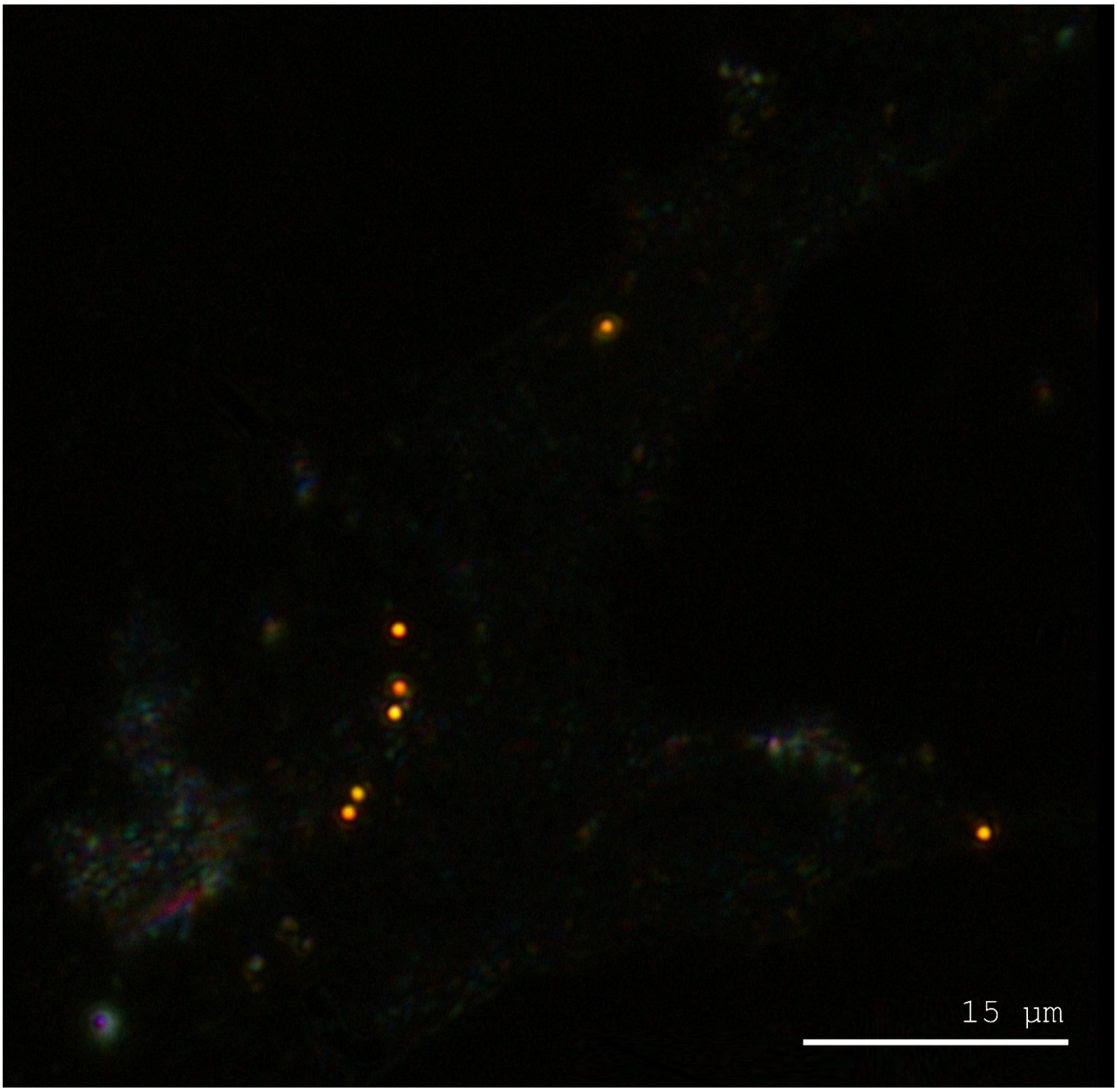

Fig. S11. Cell Imaged after 12 hours of incubation.

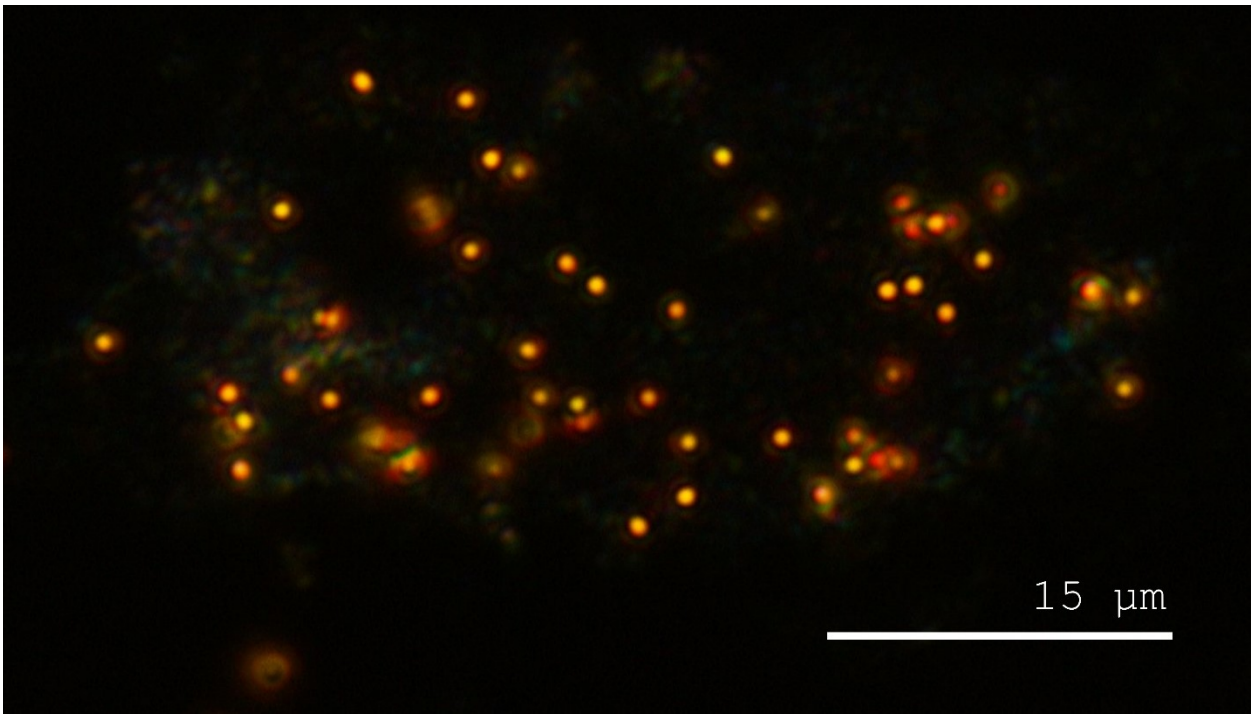

Fig. S12. Cell Imaged after 18 hours of incubation.

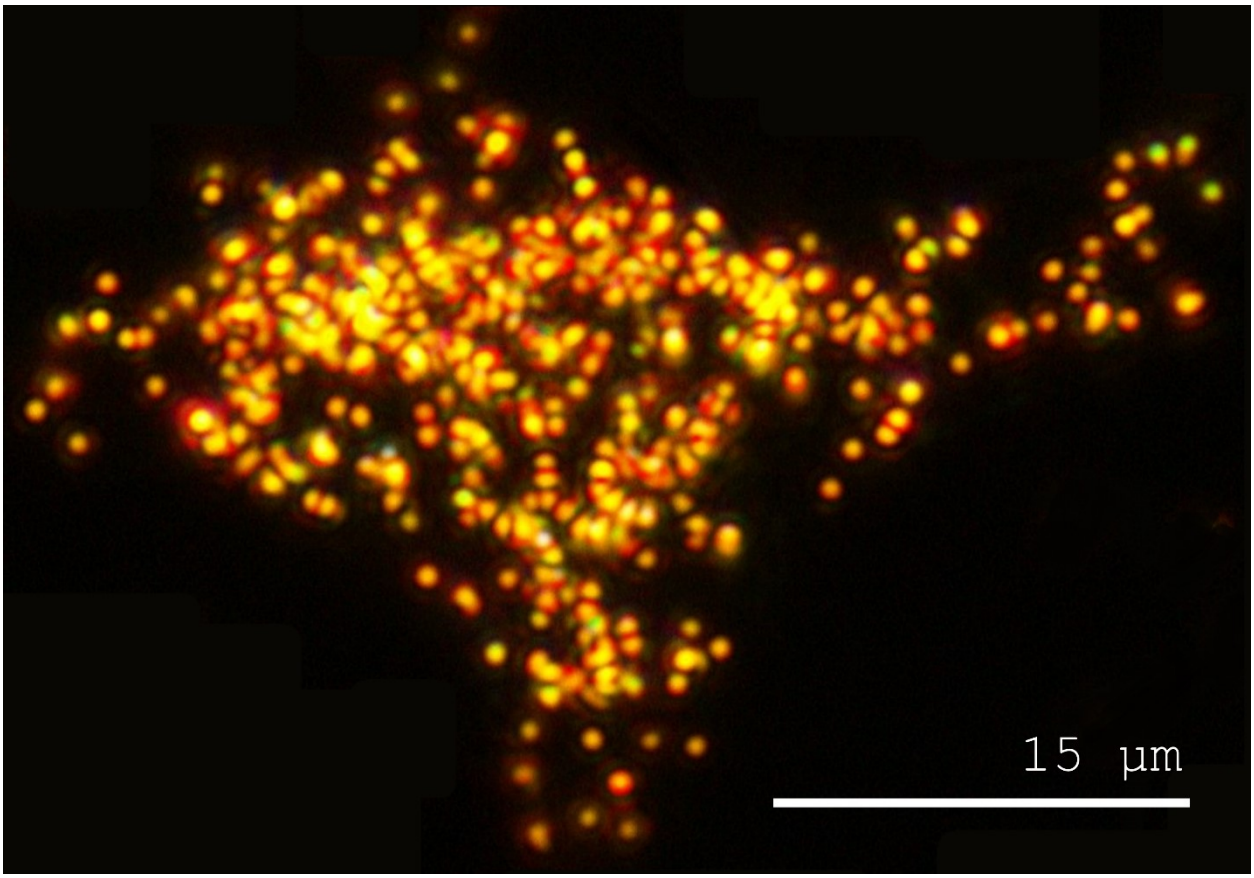

Fig. S13. Cell Imaged after 24 hours of incubation.

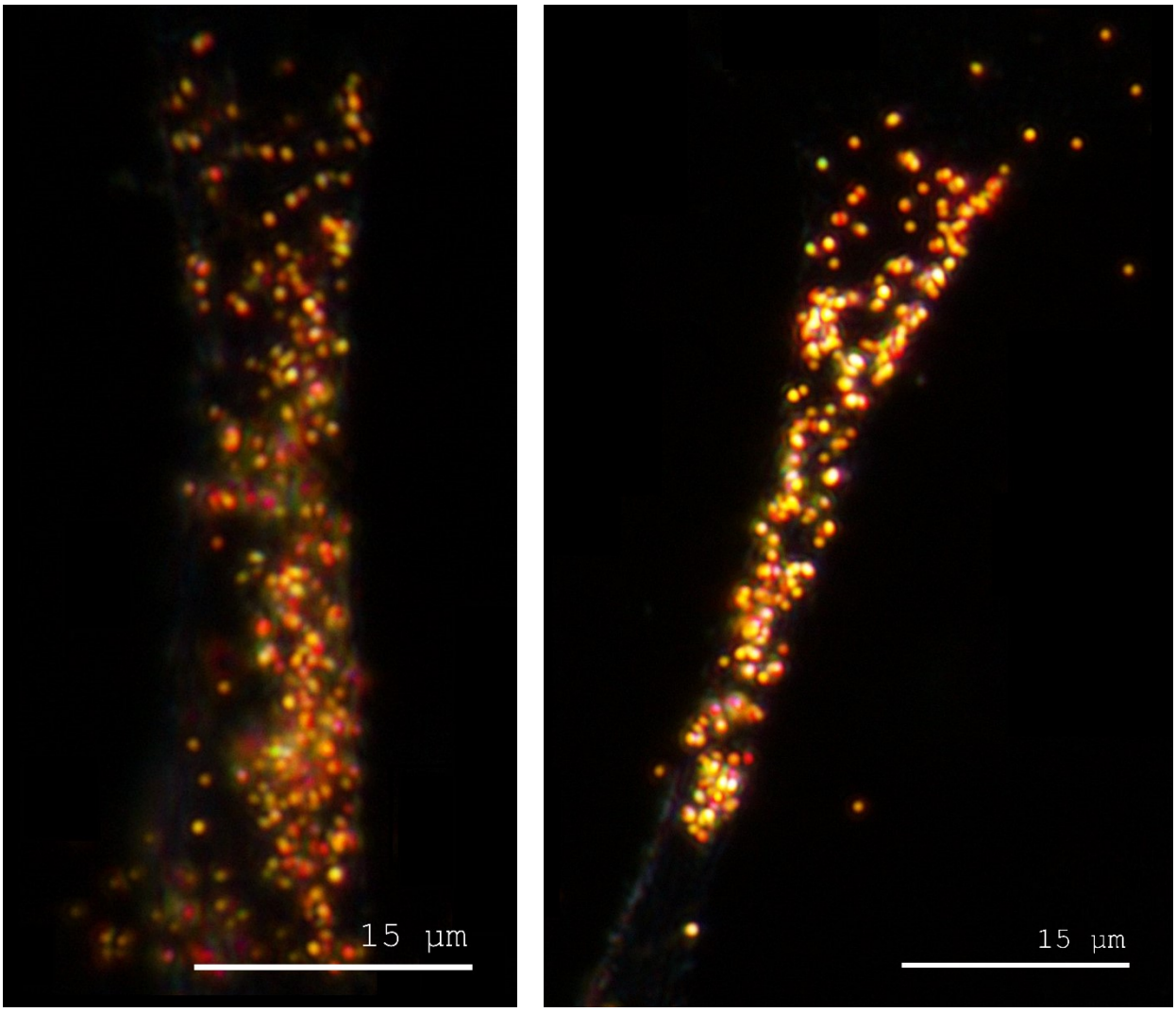

Fig. S14. Cell Imaged after 48 hours (left) and 72 hours (right) of incubation.

References

1. Palmer, A. G., Molecular aggregation characterized by high order autocorrelation in fluorescence correlation spectroscopy. *Biophysical journal* **1987,** *52* (2), 257.
2. Mohsin, A. S. M. (2015). Aggregation and uptake kinetics of gold nanoparticles in biological cells, using plasmon coupling and image correlation spectroscopy. Melbourne: Swinburne University of Technology.