Supporting Information

Multi-functional detection of extracellular vesicles with surface plasmon resonance microscopy

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Abstract

In the supporting information, we provide the details in the deep learning algorithms for the detection of single extracellular vesicles from the surface plasmon resonance microscopy images. Typical SPRM images of silica nanoparticles used for size calibration was also shown. Surface plasmon resonance microscopic video provided direct evidence on the binding and unbinding events on positively charged or CD63 antibody coated sensor surfaces.
1. Deep learning algorithms
We used a 13-layer region-based convolutional neural network (CNN), containing image input layer, 3-times repeat of convolution, rectified linear unit and maximum pooling layers, fully connected layer, softmax layer, and classification layer. The workflow is shown in Figure S1. First, we extract single silica nanoparticle images (object regions) semi-automatically as training set. These training images were normalized and rescaled before feeding them into the CNN model. The model was training iteratively, and it stops at 1000 iterations or the loss function is less than a preset threshold value. Model are saved, and the test images were fed into the model to determine the accuracy.

![Workflow of the deep learning algorithms. Key steps include image normalization and rescaling, iterative model training and accuracy test.](image)

2. Silica nanoparticle images
The single silica nanoparticles can be imaged clearly with SPRM. Figure S2 gives several examples of SPRM images of silica nanoparticles ranging from 30 nm to 1 µm.

![SPRM images of silica nanoparticles with a) 1µm, b) 600nm, c) 300nm, d) 30nm, e) 50nm.](image)

Fig. S2 SPRM images of silica nanoparticles with a) 1µm, b) 600nm, c) 300nm, d) 30nm, e)
100nm, and e) 30nm. These images are taken out of focus, showing X-shape interference patterns.

3. SPRM Videos

**Movie S1**: EVs binding on positively charge sensor surface.

**Movie S2**: EVs binding process on CD63-antibody coated sensor surface 30s after adding sample

**Movie S3**: Binding and unbinding events on CD63-antibody coated sensor surface at 550s after adding sample