Deep learning assisted quantitative detection of cardiac troponin I in hierarchical dendritic copper-nickel nanostructures lateral flow immunoassay

-Supplementary Documents

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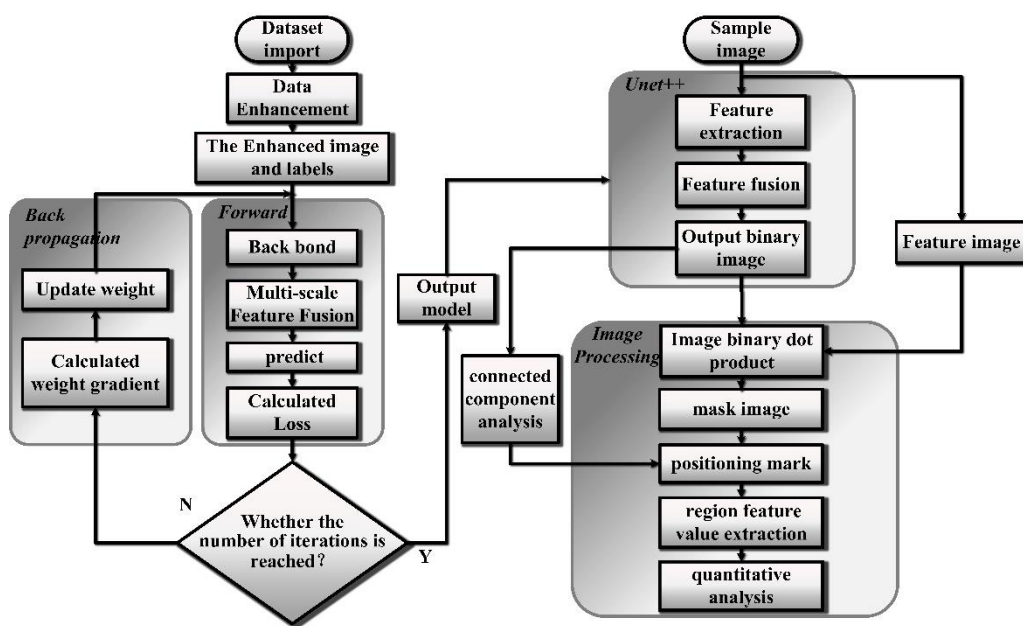*E-mail addresses: panhongcheng@glut.edu.cn*

Fig.S 1 Based on improved UNet++ for HD-nanoCu-Ni LFIA quantitative detection system flow
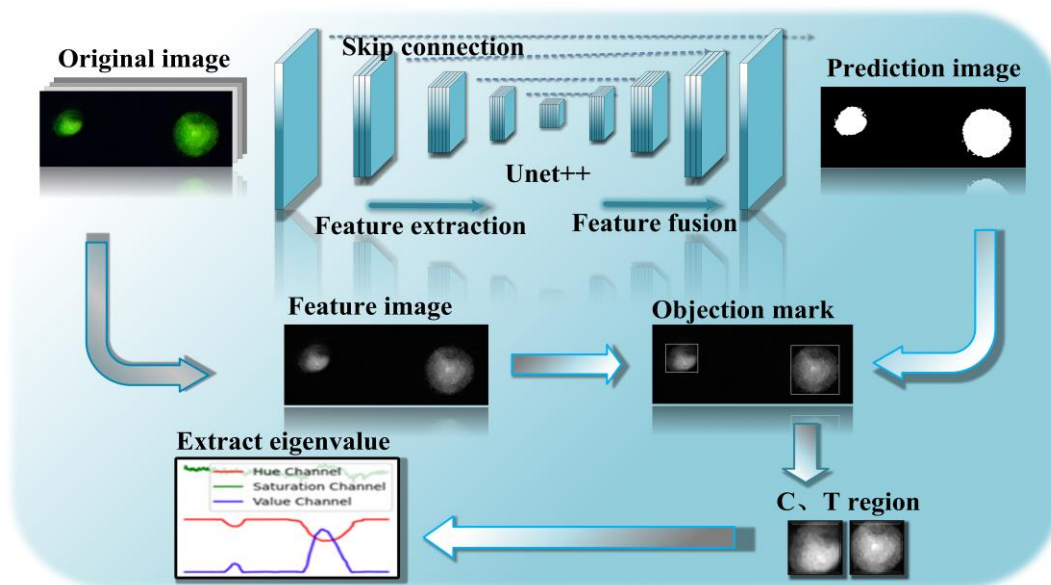

Fig.S 2 System quantitative analysis flow diagram.

A quantitative system is designed. The improved U-Net ++ model is trained to deal with the problem of sample segmentation, and the eigenvalues of the C and T regions of the sample image can be extracted by image processing technology to achieve quantitative detection. The specific process is shown in Fig.S1. The whole system is divided into training and prediction.

The left side of Fig.S1 shows the training process. The sample image data taken are imported into the network and enhanced. The enhanced sample images are successively divided by the improved UNet++ network for prediction, and the predicted images are com-pared with the labeled images to

calculate the difference between the two images, that is, the loss function. The gradient of each link of the network is calculated by the inverse derivation of the function value through the loss function, and then the weight is updated, and the prediction and error calculation of the forward channel is performed again. Through many iterations, the loss function will gradually decrease until it remains at a low level. After reaching the predetermined number of iterations, the system will save the weight of the lowest error function and generate the best model.

The right side of Fig.S1 is the quantitative analysis link, which consists of the best UNet++ model obtained from the training link and image processing technology. After the sample image is col-lected and imported into the system, image segmentation is realized through the UNet++ network. The segmented image will be output in the form of a binary graph, and the pixel values 255 and 0 correspond to the area of interest and the background area respectively. The segmentation binary image and the feature layer of the sample image are matrix-dotted to generate the mask image. Compared with the original image, the advantage of the mask im-age is that the binary map formed after segmentation is combined with the feature map. This method greatly avoids the influence of the light intensity in the non-interest region, and can make the extracted fluorescence intensity more accurate. Through the analysis of the connected domain of the binary image, the C and T reaction regions can be quickly identified, and the C and T reaction regions can be selected by marking the box in the mask image. Finally, the C and T regions were clipped and the eigenvalues were extracted respectively, and the eigenvalues representing the sample quenching reaction were calculated. The specific process effect is shown in Fig.S2.
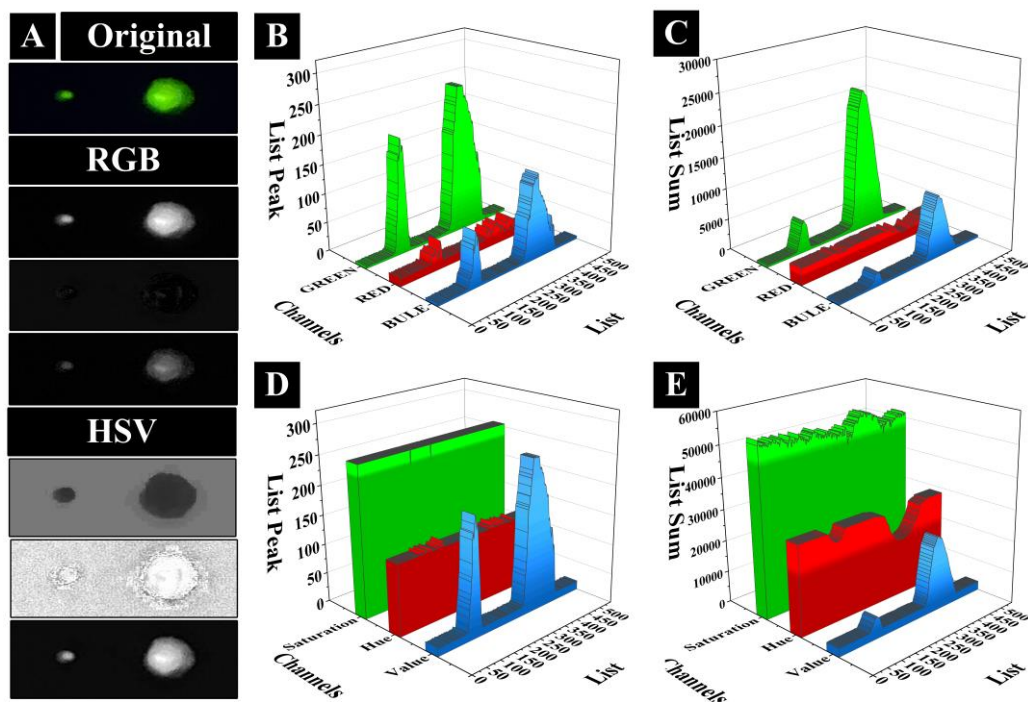
Fig.S 3 LFIA sensor sample color channel characteristic value comparison.(A): original image and RGB、HSV image; (B): RGB peak curve; (C) :RGB column sum curves; (D):HSV peak curve; E :HSV column sum curves

Different color-developing agents show different color-developing effects under excitation light. In colloid-gold immunochromatographic test papers, the gray value of C and T lines or the pixel value of R channel in RGB three channels are usually used as the characteristic value of quantitative detection. In fluorescent LFIA, the maximum value of C and T lines is used as the characteristic value to characterize the concentration of the detected substance. Unfortunately, HD-nanoCu-Ni LFIA is different from these two test strips. There is no fixed reaction region, and the fluorescence intensity is different from that of ordinary fluorescent LFIA. Therefore, we selected a sample image with a myocardial troponin I concentration of 64 ng/mL from many samples for quantitative analysis. As shown in the Original in Fig.S3A, it can be seen that this is a sample image obtained at a high concentration, in which the T-zone area of the sample has a significant gap with the area of the C zone after the immune quenching reaction. Fig.S3A also shows the visual images of the sample in RGB and HSV channels.

The experiment conducted color channel analysis on these 6 channels corresponding to the sample image. In Fig.S3, the X-axis represents the three-color channels RGB or HSV respectively, the Y-axis represents the width of the sample image, that is, the horizontal pixel

value of the image, and the Z-axis represents the column sum value or peak value respectively. The columns and values refer to the sum of all eigenvalues of the sample image at a horizontal pixel point. As shown in Fig.S3(B-C), the peak curves of RGB and HSV channels are presented, and the change of peak values of the G channel and V channel can better represent the fluorescence intensity of sample images. However, the peak surface areas of the C and T regions cannot well represent the degree of sample quenching. It can be seen that the difference between the maximum value of the T region and the peak value of the C region is not obvious even after a large degree of quenching reaction occurs. Therefore, it is unreasonable to use peak value to measure the quenching degree.

Column sum curves of sample images were analyzed, as shown in Fig.S3(CE). Through the analysis of images and channel columns and data, it is found that G and V channels can not only represent the change of fluorescence intensity in images but also the degree of fluorescence quenching in the curve area. In order to make the algorithm more generalized and compatible with fluorescent dyes other than FITC, the pixel value of the V (brightness) channel is selected as the characteristic value of fluorescence quenching.