Hyperspectral imaging analysis of photonic crystal beads array for multiplex bioassays

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Description of algorithms in the paper.

1. Reflection normalization

   The raw data is calibrated using the following equation:

   $$R_{cal}(\lambda) = \frac{I_{raw}(\lambda) - I_{dark}(\lambda)}{I_{bright}(\lambda) - I_{dark}(\lambda)}$$

   Where $R_{cal}(\lambda)$ stands for the calibrated reflectance value of each wavelength, $I_{raw}(\lambda)$ refers to the raw data intensity value of the image, $I_{dark}(\lambda)$ stands for the dark current and $I_{bright}(\lambda)$ denotes the maximum intensity value of the illumination.

2. Segmentation of the PCBs array

   (1) opening and closing operations

   The opening of image A by structuring element B, denoted $A \circ B$, is

   $A \circ B = (A \ominus B) \oplus B$

   Opening is the erosion of A by B, follow by a dilation of the result with B. Openging operations can remove small, bright details, while leaving the overall intensity levels and larger bright features relatively undisturbed.

   The closing of image A by structuring element B, denoted $A ¥ B$, is

   $A ¥ B = (A \oplus B) \ominus B$

   Closing is the dilation of A with B, follow by an erosion of the result by B. Closing can be used to fill the small holes in large bright features, and smooth the border.

   (2) The Niblack method

   $$T(x, y) = m(x, y) + k \times s(x, y)$$

   The $T(x,y)$ represents the threshold in the pixel (x,y) and it is determined using the local mean $m(x,y)$ and local standard deviation $s(x,y)$ of the region around the pixel (x,y). The k represents the adjustment coefficient.

Comparing with the threshold $T(x,y)$, the pixels can be divided into groups. Therefore, the original image is converted into a binary image.

(3) Hole filling method

Here is a fully automated procedure based on morphological reconstruction. Let $I(x,y)$ denote a binary image and suppose that we form a maker image F that is 0 everywhere, except at the image border, where it is set to $1-I$; that is,

$$F(x,y) = \begin{cases} 1 - I(x,y) & \text{if } (x,y) \text{ is on the border of } I \\ 0 & \text{otherwise} \end{cases}$$

Operation steps:

$I \rightarrow I^C \rightarrow (F \oplus B \rightarrow F \oplus B \cap I^C)$ {iterate until the current results stay the same} $\rightarrow [F \oplus B \cap I^C]^C = H$

Where I^C indicates the complementation of I, and B is a structuring element of connectivity mode 8.

Then H is a binary image equal to I with all holes filled.

(4) Statistical pattern recognition algorithm

The algorithm utilizes the differences about areas and ratio of the equivalent ellipse between the beads and the irregular spots to recognize the beads. The beads are much larger than the irregular spots, so pixels contained in one bead are much more than that in one irregular spot. Through counting pixels in every bright area, and set a threshold to compare with, it is easy to find out whether one bright area is the bead or not.