Lab 2:

Illumination Quality, Detector Sensitivity, and Refractive Index Mismatch

Lecturer: Jian Wei Tay

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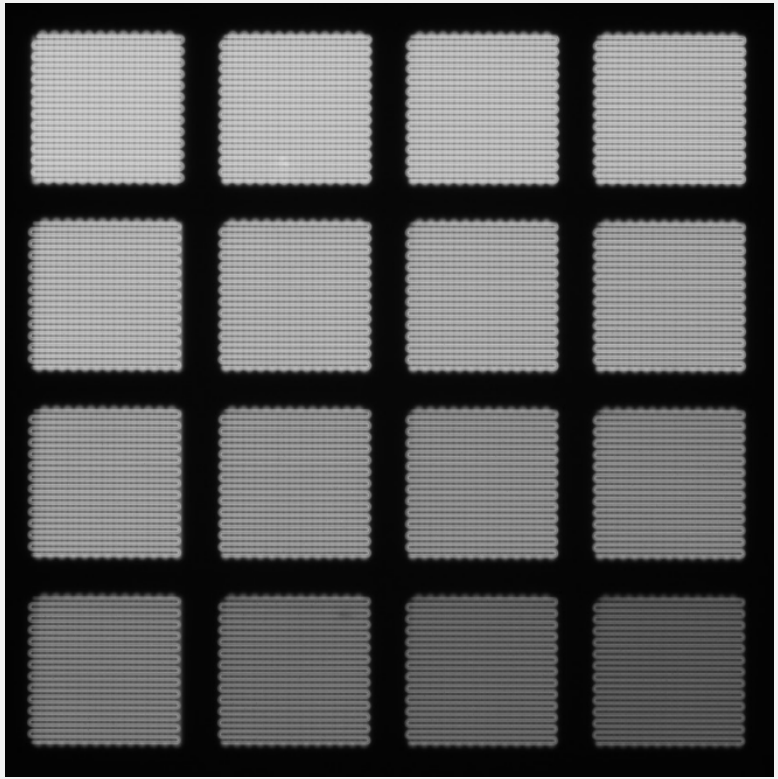
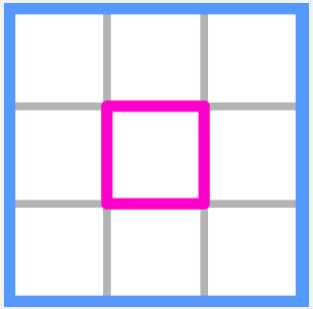
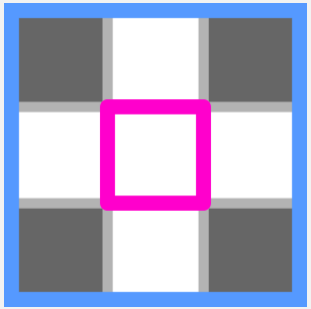
Tips for analysis



- Using this image, determine what the detector response is
- In other words, you should determine the relationship between intensity and the grayscale value of the image

The structuring element





A **structuring element** is a small logical array containing a **shape** used to probe the image

The **center (or origin)** of the structuring element is the pixel that is being probed

The **shape** is defined by `true` pixels

Use `strel` to generate structuring elements

Examples:

- `SE = strel('square', width)`
- `SE = strel('disk', radius)`
- `SE = strel('line', length, angle)`

Look at documentation for all options

Note: You can also use a logical matrix as a structuring element

The output of `strel` is a structured array `struct`

- The mask that defines the structuring element is in the field `Neighborhood`

- You can (and should) plot this to see what the structuring element looks like

```
>> imshow(SE.Neighborhood)
```

Compound morphological operations

- Opening and closing are compound morphological operations because they use the erosion and dilation operations

- Opening is erosion followed by dilation

- Closing is dilation followed by erosion

```
M = imopen(BW, SE)
```

BW = Input mask

SE = Structuring element

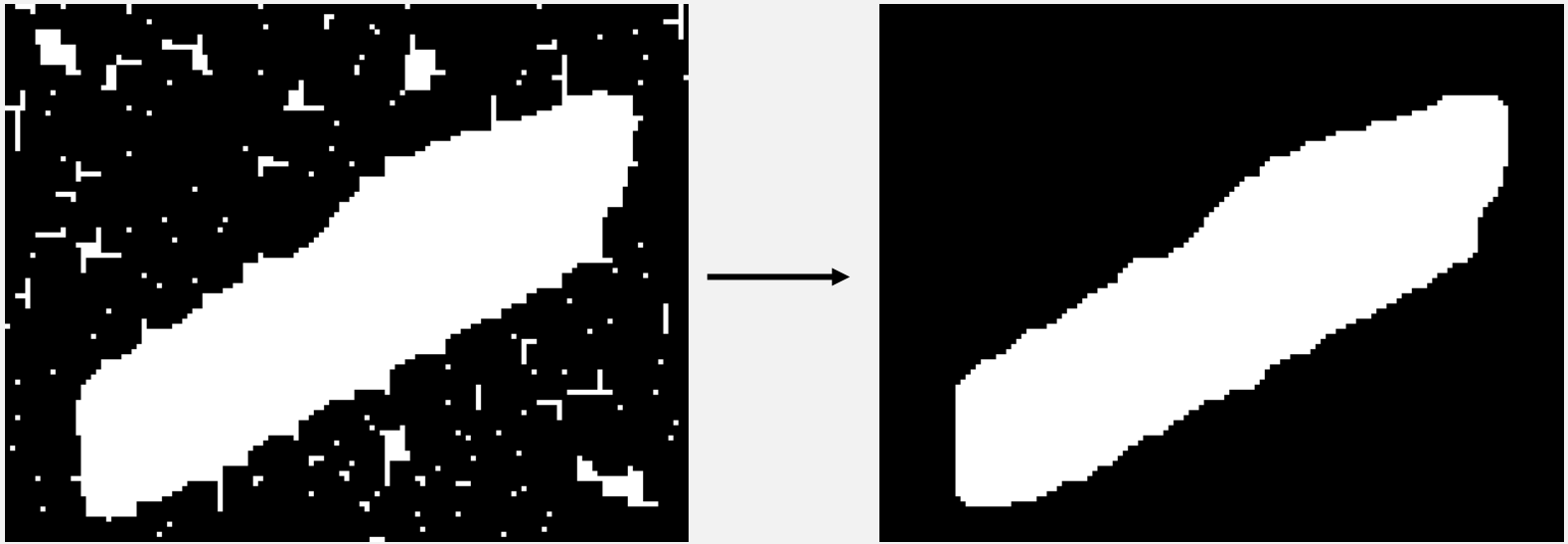
Practice

- Read in the image 'blobs.png'
- Open the image with a line structuring element, length of 5, angle 0 (horizontal line)

```
M = imopen(BW, SE)

SE = strel('line', length, angle)
```

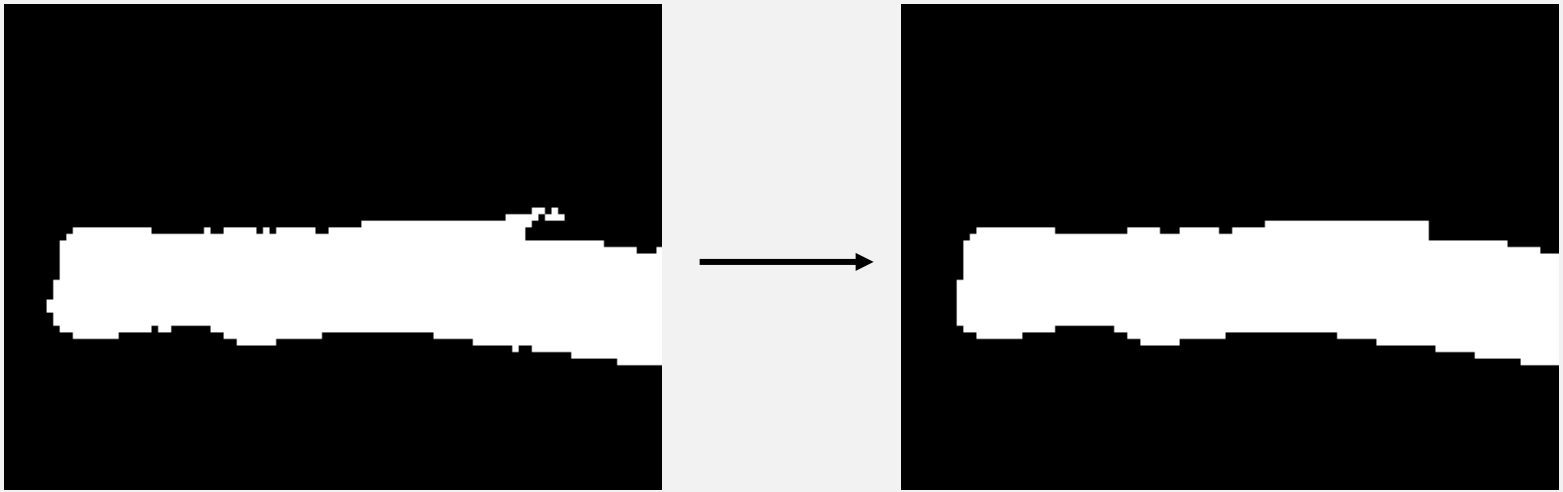
Why use opening?

- Morphological opening removes foreground objects smaller than the structuring element



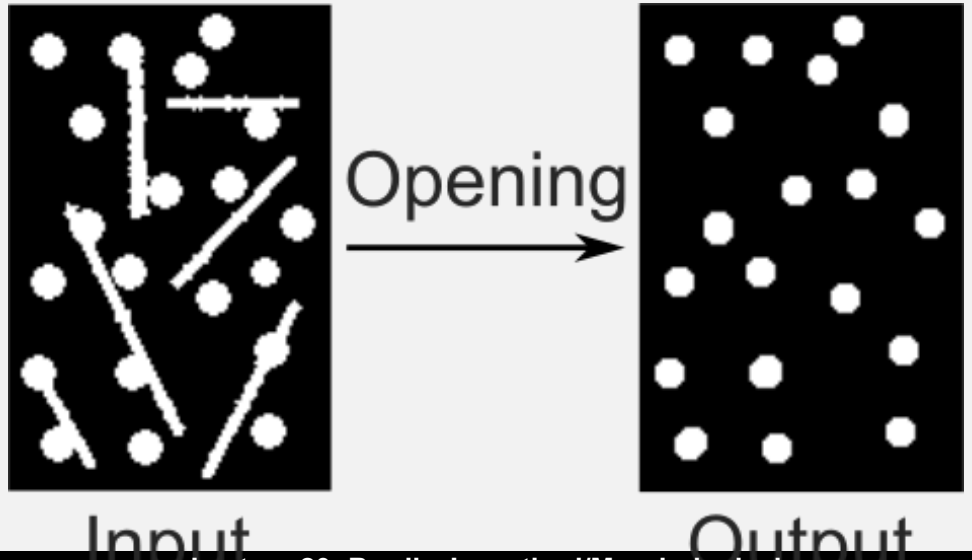
Why use opening?

- Morphological opening is useful for smoothing the edges of segmented objects



Why use opening?

- Morphological opening is useful for removing objects with a specific shape from an image



```
M = imclose(BW, SE)
```

BW = Input mask

SE = Structuring element

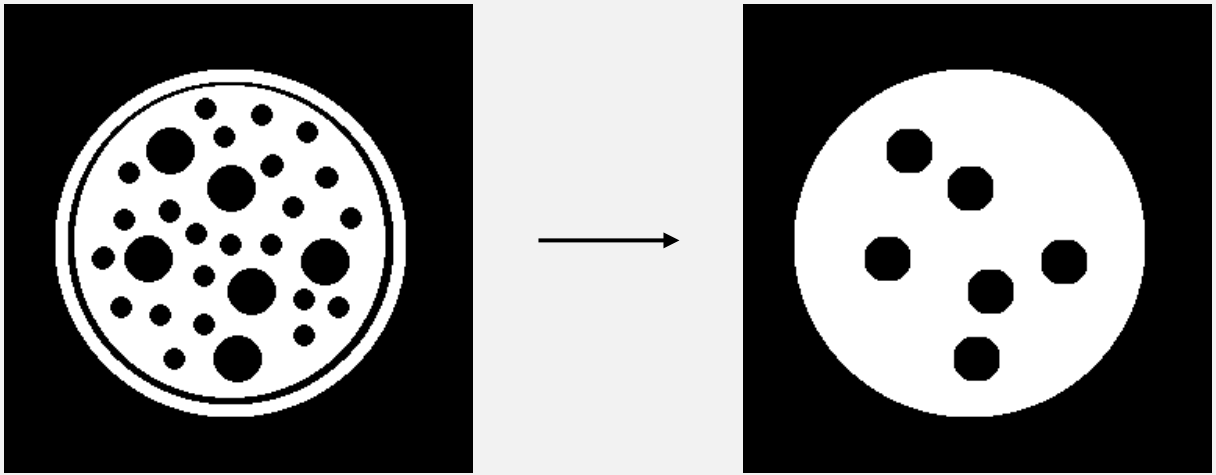
Task

- Read in the image 'blobs.png'
- Close the image with a square structuring element, width of 10

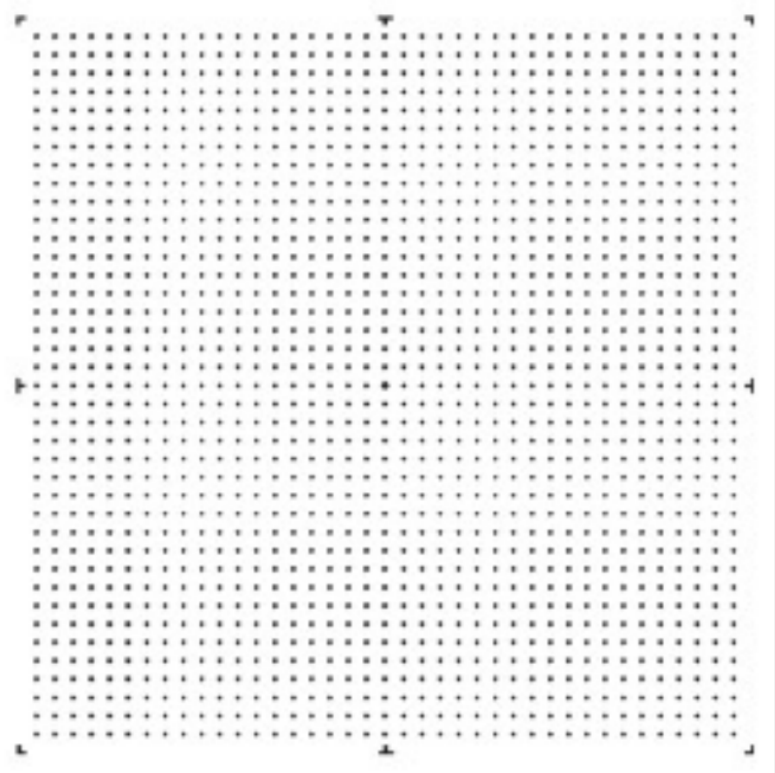
```
M = imclose(BW, SE)

SE = strel('square', width)
```

Why use closing?

- Morphological closing **fills in holes smaller than the structuring element**, while preserving the shape and size of other objects



Tips for analysis



- This pattern should be of uniformly fluorescent objects
- Using this image, determine the spatial distribution of the excitation light along camera
- One suggestion is to fit the pattern to a 2D surface

Steps for 2-D curve fitting

1. Locate and measure the average intensity for each dot
2. Define the 2D model using `fittype`
3. Fit the curve using `fit`

Note: You should be able to carry out step 1 already

2D Gaussian equation

$$z = A \exp\left(-\frac{(x-B)^2+(y-C)^2}{2D^2}\right)$$

A = amplitude

B = x-offset

C = y-offset

D = width (related to the FWHM)

Define a custom fitting model using `fittype`

model = fittype(expression, name, values)

- `expression` is a string of the model equation

- You might need a couple of additional arguments:
  - `'dependent'` – string that specifies the dependent (output) variable name
  - `'independent'` – string that specifies the independent (input) variables

2D Gaussian equation

$$z = A \exp\left(-\frac{(x-B)^2+(y-C)^2}{2D^2}\right)$$

- In this equation:
  - z is the dependent variable
  - x and y are the independent variables

Work with a partner to write the command that declares this model

Using `fit` to fit to a surface

```
fitObj = fit([x, y], z, model)
```

- x and y need to be column vectors specifying the x- and y- coordinates of the measured data (i.e., intensity of the spots)

Adding an initial guess

```
curve = fit([X, Y], Z, 'StartPoint', p0 );
```

`p0` is a matrix with an initial guess for each coefficient. For our model, `p0 = [A B C D]`.

Making a guess that is close to the actual values will improve accuracy of the fit.

You only need to do this if the fitting is poor.