MCDB/BCHM 4312 & 5312 – Quantitative Optical Imaging

Lecture 8:

Performing calculations with matrices

Lecturer: Jian Wei Tay

Date: 10 September 2021

Image Analysis Homework Clarifications

- Homework is now provided as a Word document
- You should download and type your answers in the document, then save as PDF
- Code MUST be correct MATLAB syntax - incorrect syntax will not be accepted.
- Export images by displaying the image in MATLAB, then clicking File > Save As, or by using the `imwrite` function. Include the image in the Word document in the space provided.
- Upload the answers for the image analysis section as a separate file from the microscopy section. This helps us with grading on Canvas.

Learning objectives

- Statistical functions
- Generating a normalized image intensities
- Understand the difference between array and matrix operations
- Array operators in MATLAB

Statistical functions

- Useful statistical functions:
  - `mean`
  - `min`
  - `max`
  - `std`
  - `median`

Practice

- Enter the following commands to create a (pseudo-) random matrix:

```
>> rng (1)
>> M = randi(25, 4)
```

Practice

- Compute the mean of the matrix M

```
>> mean(M)
```

- What is the meaning of the results?

Question

- Which of the following commands will return the mean of all the elements in M?

```
A. mean(M)
B. mean(M, 'all')
C. mean(M, [], 'all')
D. mean(M, :)
```

Calculating the mean along a dimension

- From the help text of `mean`:

  ```
  mean(X,DIM) takes the mean along the dimension
  DIM of X.
  ```

- From Lecture 4: MATLAB defines rows as `DIM = 1` and columns as `DIM = 2`

Question

- Which of the following commands returns the mean of each row?

```
A. mean(M)
B. mean(M, 1)
C. mean(M, 2)
D. mean(M, 'row')
```

Questions?

Application: Normalizing images

- It is usually difficult to take microscope images that span the full *dynamic range* (bit range)

- When preparing images for display or publication, it is often helpful to normalize the intensity to increase image contrast (difference between the light and dark regions of the image)

- Also called "contrast stretching"

Practice

- Read in and display the image `l8_low_contrast.tif`

- Try writing the image to a PNG file:

```
>> imwrite(I, 'l8_output.png')
```

- Open the file in your image viewer and verify that the cell is hard to see

Calculating contrast

- The (global) contrast of an image is defined as

$$C = I_{max} - I_{min}$$

where I_{max} is the maximum image intensity and I_{min} is the maximum image intensity

Note: What contrast value makes the "best" image is somewhat subjective… but a higher contrast is generally easier to see

Question

- Using the functions `max` and `min`, compute the global contrast of the image `l8_low_contrast.tif`

Linear normalization equation

$$I_{norm} = \frac{I - I_{min}}{I_{max} - I_{min}}$$

where I_{min} is the minimum intensity of the image and I_{max} is the maximum intensity of the image.

This equation will return values of I_{norm} between 0 and 1.

Practice

- Apply linear normalization to increase the image contrast

$$I_{norm} = \frac{I - I_{min}}{I_{max} - I_{min}}$$

Remember to check data types

- From lecture 6: Image data type is an unsigned integer. What happens when we divide by the maximum intensity?

Converting between data types

- Use the appropriate function:
  - `double` – converts a numeric data type to double-precision
  - `uint16` – converts a numeric data type to unsigned 16-bit integer

Note: Look back at Lecture 6 for the description of these data types

Practice

- Taking into account data types, apply linear normalization to increase the image contrast

$$I_{norm} = \frac{I - I_{min}}{I_{max} - I_{min}}$$

Practice

- Write the normalized image to a PNG file

- Open the image with an image viewer. Check that the cell is now visible.

Question

- How does the contrast of this image compare with the original? **Hint:** Need to multiply normalized image values by 65535 to get comparable values.

Questions?

Image mishandling

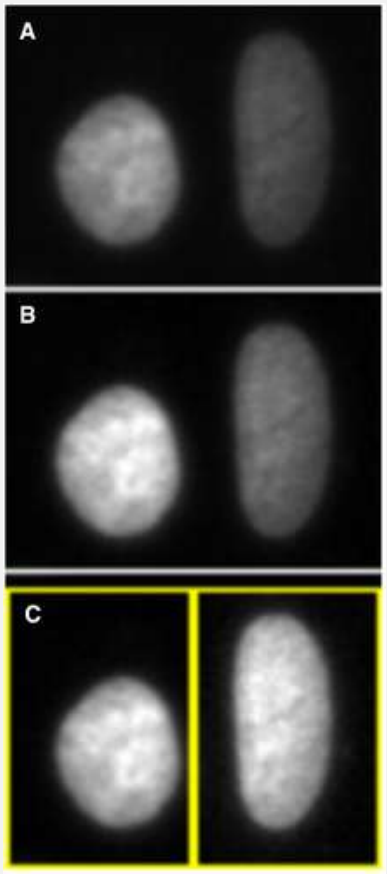
- By modifying image values, it is important to note that this constitutes data manipulation

- When is it appropriate to use contrast stretching?

… Enhancing the contrast of image data is generally acceptable if (i) it is applied for segmentation or to create the figures, (ii) no intensity quantification is involved after the operation, (iii) it is applied equally to all conditions, and (iv) it is clearly stated in the manuscript

Reproducible image handling and analysis, Miura and Nørrelykke, EMBO J (2021) 40:e105889

https://doi.org/10.15252/embj.2020105889

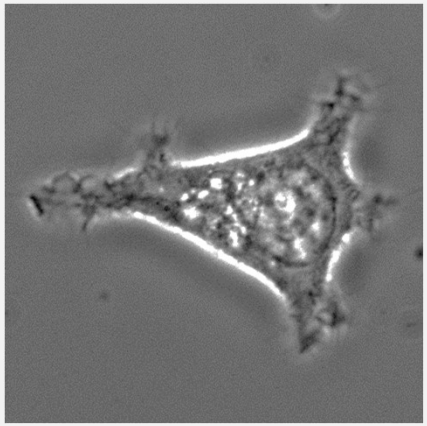


Original

Contrast enhancement applied to whole image

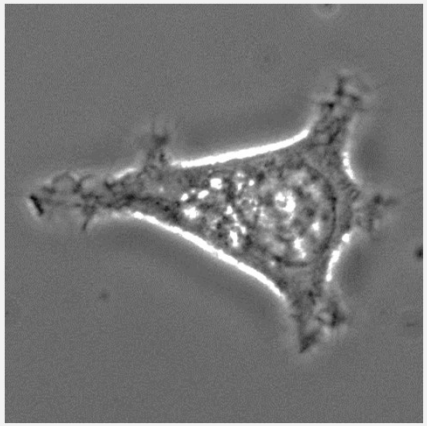
Contrast enhancement applied to cells individually

Never use JPEG or GIFs for analysis



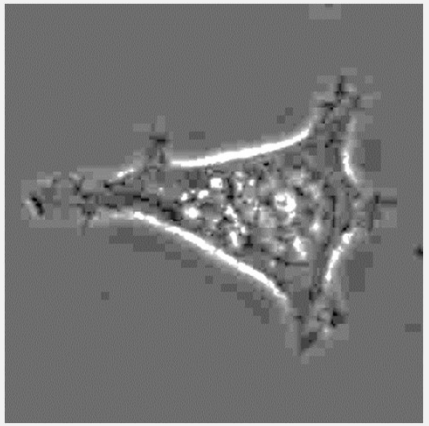
Original
Uncompressed TIFF

535 kB



40%
JPEG compression

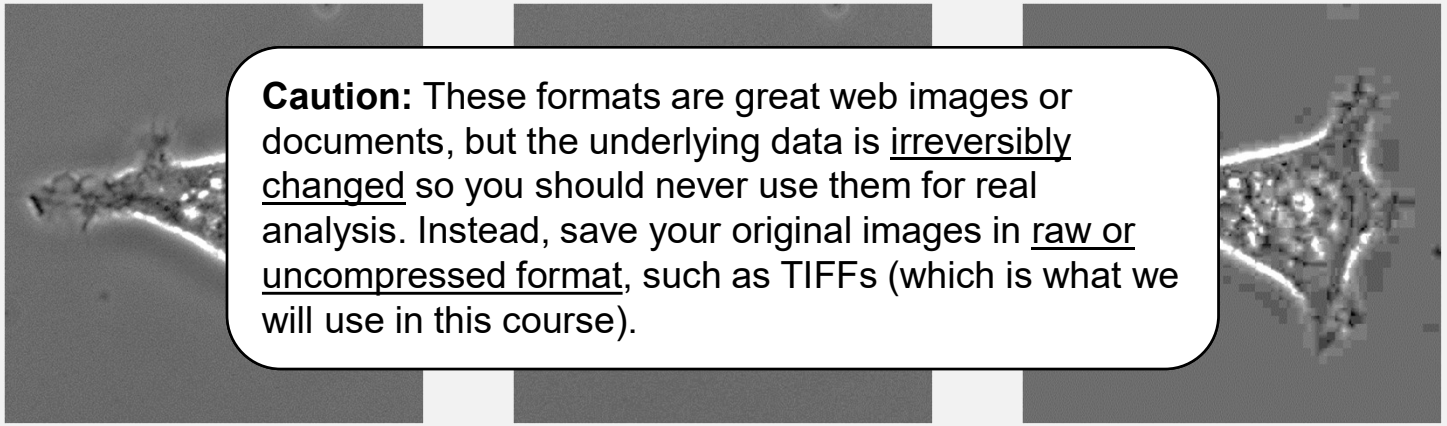
19.8 kB



95%
JPEG compression

10 kB

Never use JPEG or GIFs for analysis



Caution: These formats are great web images or documents, but the underlying data is irreversibly changed so you should never use them for real analysis. Instead, save your original images in raw or uncompressed format, such as TIFFs (which is what we will use in this course).

Original Uncompressed TIFF	40% JPEG compression	95% JPEG compression
535 kB	19.8 kB	10 kB

Questions?

Array vs Matrix Operations

- Array operations are carried out between elements in a matrix

- Matrix operations carry out linear algebra operations

Array and matrix operators in MATLAB

Operation	Array operator	Matrix operator
Multiplication	`.*`	`*`
Division	`./`	`/`
Power	`.^`	`^`
Addition	`+`	`+`
Subtraction	`-`	`-`

Note: The addition and subtraction operators are the same for array and matrix operations. `.+` and `.-` do not exist.

Example of array multiplication

$$\begin{bmatrix} 10 & 3 \\ 8 & 12 \end{bmatrix} * \begin{bmatrix} 8 & 5 \\ 15 & 1 \end{bmatrix} = \begin{bmatrix} 10 \times 8 & 3 \times 5 \\ 8 \times 15 & 12 \times 1 \end{bmatrix}$$

$$= \begin{bmatrix} 80 & 15 \\ 120 & 12 \end{bmatrix}$$

Practice

- Declare the following matrices as variables `A` and `B`

$$\begin{bmatrix} 10 & 3 \\ 8 & 12 \end{bmatrix}$$

A

$$\begin{bmatrix} 8 & 5 \\ 15 & 1 \end{bmatrix}$$

B

Practice

- Type the following command to perform an array multiplication

```
>> A .* B
```

Example of matrix multiplication

$$\begin{bmatrix} 10 & 3 \\ 8 & 12 \end{bmatrix} * \begin{bmatrix} 8 & 5 \\ 15 & 1 \end{bmatrix} = \begin{bmatrix} (10 \times 8) + (3 \times 15) & (10 \times 5) + (3 \times 1) \\ (8 \times 8) + (12 \times 15) & (8 \times 5) + (12 \times 1) \end{bmatrix}$$

$$= \begin{bmatrix} 125 & 53 \\ 244 & 52 \end{bmatrix}$$

Practice

- Type in the following command to perform a matrix multiplication

```
>> A * B
```

Operations between a matrix and a scalar

- Do you need an array operator between a matrix and a scalar?
  - `A * 2`
  - `A / 5`
  - `1 / A`

Questions?

Note: We'll see more uses of array operations next week