Project

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1 Image processing assignment #1

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1.1 Image Loading

In the first part of the assignment, we will use the CLI tool tiffinfo to investigate the handed-out images.

[1]: !tiffinfo -D data/imgset1/Kidney1.tif

```
TIFF Directory at offset 0x866312 (d3808)
```

Subfile Type: (0 = 0x0)

Image Width: 1120 Image Length: 2508 Image Depth: 1

Tile Width: 240 Tile Length: 240

Bits/Sample: 8

Compression Scheme: JPEG

Photometric Interpretation: RGB color

Samples/Pixel: 3

Planar Configuration: single image plane

ImageDescription: Aperio Image Library v12.0.15

18288x40236 [0,100 17927x40136] (240x240) -> 1120x2508 JPEG/RGB Q=92

JPEG Tables: (574 bytes)

[2]: # !tiffinfo -D data/imqset1/*

[Output has been removed for brevity]

Evidently, this tool provides metadata about TIFF images, such as their dimensions, compression type, bit depth, etc. The obtained information shows the structure and properties of each image.

Some observations:

- TMA2-v2.tif contains subfiles with different dimensions (3102x3102 and 1551x1551), it is a pyramid TIFF.
- Kidney2_RGB2_20x.svs has subfiles with different dimensions, it's not clear what they represent.
- All images have the tile size of 240x240 pixels.
- Some images are compressed using LZW, while others are uncompressed JPEG.

- All images are 8-bit, which means they have 256 levels of intensity.
- Some images have 1 sample per pixel (grayscale), while others have 3 samples per pixel.
- Of the images with 3 samples per pixel, some have RGB color space, while others have YCbCr color space.

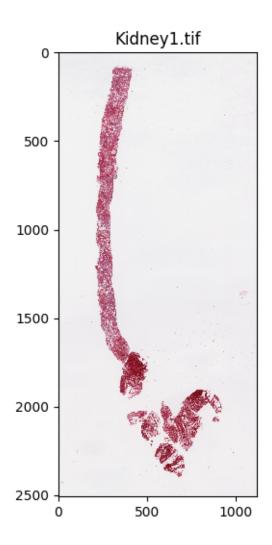
1.1.1 Image loading

The next task involves loading images using the libtiff library. We will load whole images and sub-rectangles by combining tiles read using the read_one_tile function from the TIFF library.

Whole image First, we will load the whole image Kidney1.tif and display it.

```
[3]: import matplotlib.pyplot as plt
from libtiff import TIFF

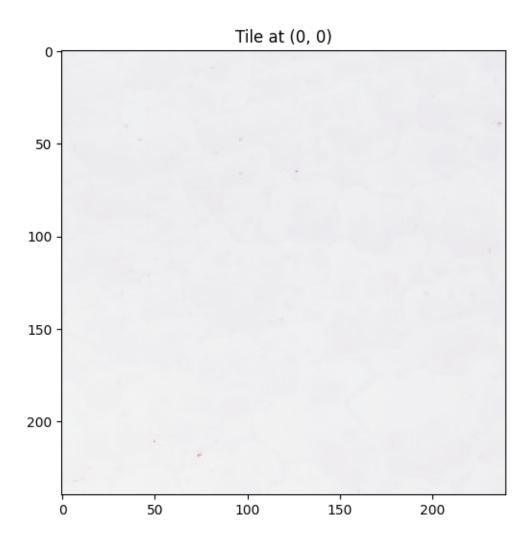
plt.figure(figsize=(6, 6))
plt.imshow(
    TIFF.open("data/imgset1/Kidney1.tif").read_image(),
    interpolation="nearest",
    vmin=0,
    vmax=255,
)
plt.title("Kidney1.tif")
plt.show()
```



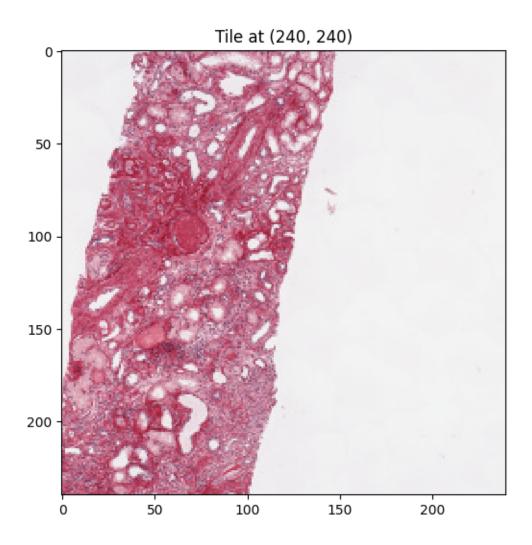
Sub-rectangles Next, we will load select sub-rectangles of the image Kidney1.tif and display them. We will load the following sub-rectangles:

- Top-left corner (0, 0) with dimensions 240x240.
- Second column, second row (240, 240) with dimensions 240x240.

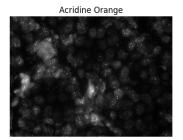
```
[4]: plt.figure(figsize=(6, 6))
  plt.imshow(
        TIFF.open("data/imgset1/Kidney1.tif").read_one_tile(0, 0),
        interpolation="nearest",
        vmin=0,
        vmax=255,
)
  plt.title("Tile at (0, 0)")
  plt.show()
```

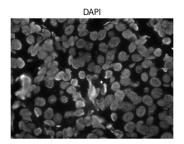


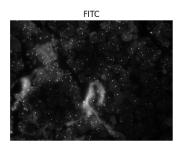
```
[5]: plt.figure(figsize=(6, 6))
plt.imshow(
    TIFF.open("data/imgset1/Kidney1.tif").read_one_tile(240, 240),
    interpolation="nearest",
    vmin=0,
    vmax=255,
)
plt.title("Tile at (240, 240)")
plt.show()
```



We have defined helper functions display_grayscale_images, display_rgb_images to display multiple images in a grid.

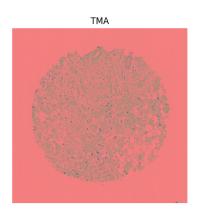












We visualized these images using matplotlib. One possible anomaly is that TMA2-v2.tif is displayed with a pink background. This is possibly due to the fact that the image uses YCbCr color space.

1.1.2 Image combination

Now we shall combine three fluorescence images into one contiguous RGB image and save it to disk.

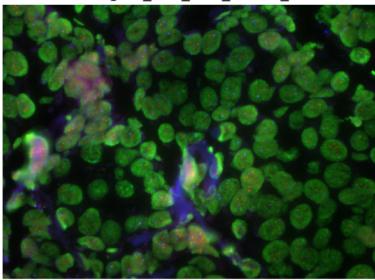
The combination process involved:

- Loading each grayscale image.
- Stacking the images along the third axis to form an RGB image.

- Red channel: data/imgset1/Region_001_FOV_00041_Acridine_Or_Gray.tif
- Green channel: data/imgset1/Region_001_FOV_00041_DAPI_Gray.tif
- Blue channel: data/imgset1/Region_001_FOV_00041_FITC_Gray.tif
- Saving the resulting RGB image to disk.

Combined image shape: (1024, 1392, 3) Saved transformed image to output.tif

Combined Region_001_FOV_00041_* Channels



1.2 Intensity Transformations

In the second part of the assignment, we will apply intensity transformations to the images. We will use the following transformations:

- Power-law
- Piece-wise linear transformations
 - Histogram stretching
 - Thresholding

- Histogram calculations
- Histogram normalization

1.2.1 Power-law

The power law transformation is given by the formula $I_{\text{out}} = I_{\text{in}}^{\gamma}$, where γ is a constant. This transformation is often used to enhance contrast or adjust brightness.

Implementation:

- Input: An 8-bit grayscale image and a value for γ
- Process: Apply the power law transformation to each pixel's intensity value. Intermediate calculations are done using float arithmetic before converting the result back to uint8, because the result may well exceed the 8-bit range.
- Output: The transformed image.

Parameter:

- < 1: Enhances darker regions of the image.
- > 1: Enhances brighter regions of the image.

TIFFFetchNormalTag: Warning, ASCII value for tag "ImageDescription" does not end in null byte.

Powerlaw image shape: (571, 482)

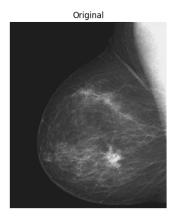
Saved transformed image to output1.tif

TIFFFetchNormalTag: Warning, ASCII value for tag "ImageDescription" does not end in null byte.

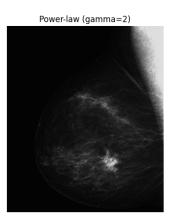
Powerlaw image shape: (571, 482)

Saved transformed image to output2.tif

TIFFFetchNormalTag: Warning, ASCII value for tag "ImageDescription" does not end in null byte.







As you can see, the power-law transformation with =0.5 brightens the dark regions, while =2 darkens the darker regions.

Piece-wise Linear transformations

Piece-wise linear transformations involve applying different linear transformations to different ranges of intensity values. Here we shall demonstrate histogram stretching and thresholding.

Histogram stretching Histogram stretching enhances contrast by expanding the range of intensity values.

Implementation:

- Expand the range of intensity values to enhance contrast.
 - Input: An 8-bit grayscale image.
 - Process: Calculate the minimum and maximum intensity values in the image. Then, apply the following transformation to each pixel's intensity value: $I_{\rm out} = \frac{I_{\rm in} - I_{\rm min}}{I_{\rm max} - I_{\rm min}} \times 255$.

 * Intermediate calculations are done using float arithmetic, though using uint16
 - would also be possible by performing multiplication before division.
 - Output: The transformed image.

```
[13]: | python python/main.py histogram-stretching data/imgset2/
        \rightarrowFig0316\(2\)\(2nd_from_top\).tif
```

TIFFFetchNormalTag: Warning, ASCII value for tag "ImageDescription" does not end in null byte.

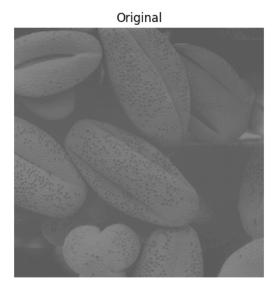
Min value: 91 Max value: 138

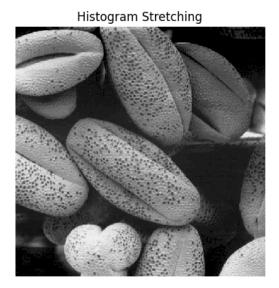
Stretched image shape: (500, 500) Saved transformed image to output.tif

```
[14]: display_grayscale_images(
              "data/imgset2/Fig0316(2)(2nd_from_top).tif",
```

```
"output.tif",
],
titles=["Original", "Histogram Stretching"],
figsize=(10, 5),
)
```

TIFFFetchNormalTag: Warning, ASCII value for tag "ImageDescription" does not end in null byte.





As you can see, the histogram stretching transformation strongly enhanced the contrast of the image by expanding the range of intensity values.

Thresholding Thresholding sets pixel values to a minimum or maximum level if they fall above or below a certain threshold.

Implementation:

- Set pixel values to min/max level if they fall above or below a certain threshold.
 - Input: An 8-bit grayscale image and a threshold value.
 - Process: Set pixel values below the threshold to 0 and those above the threshold to 255.
 Intermediate calculations are done using int arithmetic because the result is within the 8-bit range.
 - Output: The thresholded image.

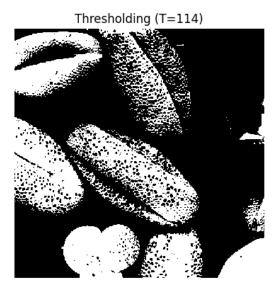
```
[15]: | !python python/main.py thresholding data/imgset2/Fig0316\(2\)\(2nd_from_top\).
```

TIFFFetchNormalTag: Warning, ASCII value for tag "ImageDescription" does not end in null byte.

Thresholded image shape: (500, 500) Saved transformed image to output.tif

TIFFFetchNormalTag: Warning, ASCII value for tag "ImageDescription" does not end in null byte.





As you can see, thresholding with a threshold value of 114 resulted in a binary (black & white) image with only two intensity levels.

1.2.3 Histogram calculation

A histogram represents the frequency distribution of intensity values in an image.

Implementation:

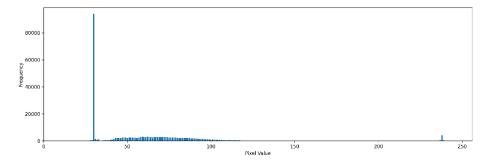
- Input: An 8-bit grayscale image.
- Process: Count the occurrences of each intensity value (0-255) and store them in a frequency array.
- Output: The histogram array.

TIFFFetchNormalTag: Warning, ASCII value for tag "ImageDescription" does not end in null byte.

```
Histogram shape: (256,)
Histogram: [
                  0
                                0
                                       0
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0
     0
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                         12
                               704
                                      460 93879
                                                  1526
                                                         1029
                                                                1444
                                                                        254
                                                                               322
                 499
                        579
                                                                2288
                                                                       2226
   399
          448
                               778
                                     1056
                                           1454
                                                  2013
                                                         2143
                                                                              2455
  2400
         2389
                2309
                      2351
                             2354
                                    2383
                                           2347
                                                  2253
                                                         2306
                                                                2651
                                                                       2883
                                                                              3060
  2944
         2934
                3042
                      2951
                              2954
                                    2627
                                           2839
                                                  2898
                                                         2935
                                                                2894
                                                                       2764
                                                                              2833
  2861
         2938
                2714
                      2530
                              2573
                                    2637
                                           2600
                                                  2409
                                                         2213
                                                                2337
                                                                       2317
                                                                              2270
  2162
         2011
                2065
                      2088
                              1858
                                     1922
                                           1953
                                                  1865
                                                         1715
                                                                1500
                                                                       1566
                                                                              1467
  1373
         1331
                1197
                                     1069
                                             883
                                                   893
                                                                 799
                                                                        779
                                                                               702
                      1144
                              1063
                                                          842
   681
                 592
                        584
                               515
                                      499
                                             471
                                                   438
                                                          426
                                                                        339
                                                                               378
          613
                                                                 412
   377
          363
                 348
                        307
                                             287
                                                   258
                                                                               224
                               307
                                      286
                                                          258
                                                                 256
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   217
          210
                 226
                        228
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                 155
                        118
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   145
          168
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                                      181
                                             171
                                                   201
                                                          294
                                                                 690
                                                                       4390
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   119
           57
                  33
                         27
                                13
                                       11
                                               9
                                                      4
                                                             4
                                                                    0
                                                                          0
                                                                                 2
     0
            0
                   0
                          1]
```

Saved histogram to histogram.tif

Histogram of Fig0304(a)(breast_digital_Xray) intensity values



You can see the distribution of intensity values. There is a sharp peak around 30, with few values below 30. There is a small peak around 240, with few values above 240. The rest of the values are mostly distributed between 40 and 110.

1.2.4 Histogram normalization

Histogram normalization adjusts the intensity values to use the full range of possible values (0-255).

Compared with Piece-wise transformation, which manually defines transformations for specific intensity ranges, histogram normalization automatically spreads the intensity values to cover the full range.

In our implementation the number of unique intensity values does not change, because any pixels with the same intensity value will be mapped to the same new value.

Implementation:

- Input: An 8-bit grayscale image.
- Process: Calculate the cumulative distribution function (CDF) from the histogram, then use it to map the original intensities to new values. Intermediate calculations are done using float arithmetic before converting the result back to uint8, though it would also be possible to use uint8 and avoid casting.
- Output: The normalized image.

```
[19]: | python python/main.py histogram-normalization data/imgset2/

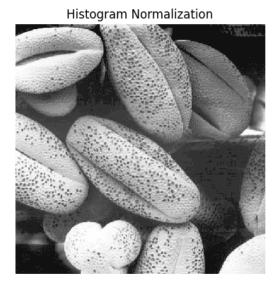
Fig0316\(2\)\(2nd_from_top\).tif
```

TIFFFetchNormalTag: Warning, ASCII value for tag "ImageDescription" does not end in null byte.

Normalized image shape: (500, 500) Saved transformed image to output.tif

TIFFFetchNormalTag: Warning, ASCII value for tag "ImageDescription" does not end in null byte.

Original



1.3 Spatial Filtering and Multistep Image Processing

We will work with 8-bit grayscale images and intermediate results stored as floating-point images.

1.3.1 Image conversion

We have defined two classes in image_classes.py:

- Image8Bit for 8-bit images
- ImageFloat for floating-point images

In order to avoid saving floating-point images to the disk (which is not allowed) ImageFloat does not have a save method while Image8Bit does.

Image8Bit has a method to_float that converts the image to floating-point format by dividing the pixel values by 255.0, while ImageFloat has a method to_8bit that converts the image to 8-bit format by multiplying the pixel values by 255.0 and clipping them to the 0-255 range.

1.3.2 Image blurring

Implementation:

- Input: An 8-bit grayscale image.
- Process: Apply Averaging convolution to the image.
 - The average filter is a simple low-pass filter that replaces each pixel's intensity value with the average of the pixel values in its neighborhood, as defined by a square kernel of a given size (kernel-size parameter).
- Output: The blurred image.

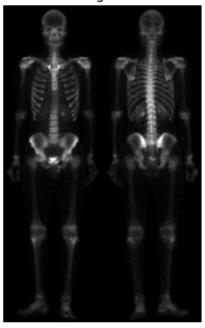
Parameters:

• kernel-size: The size of the square kernel used for averaging. A larger kernel size results in more blurring.

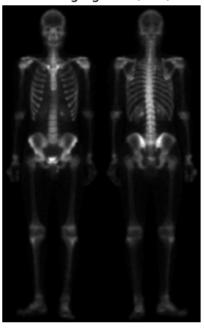
```
[21]: | python python/main.py blur data/imgset3/Fig0343\(a\)\(skeleton_orig\).tifu
```

Blurred image shape: (800, 500) Saved image to output.tif

Original



Averaging blur (5x5)



As you can see, the right image is blurred.

1.3.3 Image Sharpening

Implementation:

- Input: An 8-bit grayscale image.
- Process:
 - Laplacian: Apply the axial Laplacian filter to the image. Add the result (scaled by $\tt c)$ to the original image.

- Unsharp: Subtract the blurred image from the original image and add the result to the original image.
 - * The blurred image is obtained by applying the average filter to the original image.
- Output: The blurred image.

Parameters:

- method: Either laplacian or unsharp.
- c: Sharpening factor. A larger value results in more sharpening.

Laplacian

```
[71]: !python python/main.py sharpen laplacian data/imgset3/

Fig0338\(a\)\(blurry_moon\).tif --c 4
```

```
TIFFReadDirectory: Warning, Unknown field with tag 34016 (0x84e0) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34017 (0x84e1) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34018 (0x84e2) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34019 (0x84e3) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34020 (0x84e4) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34021 (0x84e5) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34022 (0x84e6) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34023 (0x84e7) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34024 (0x84e8) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34025 (0x84e9) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34026 (0x84ea) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34027 (0x84eb) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34028 (0x84ec) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34029 (0x84ed) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34030 (0x84ee) encountered.
_TIFFVSetField: data/imgset3/Fig0338(a)(blurry_moon).tif: Null count for "Tag
34022" (type 1, writecount -3, passcount 1).
_TIFFVSetField: data/imgset3/Fig0338(a)(blurry_moon).tif: Null count for "Tag
34025" (type 1, writecount -3, passcount 1).
_TIFFVSetField: data/imgset3/Fig0338(a)(blurry_moon).tif: Null count for "Tag
34026" (type 1, writecount -3, passcount 1).
Sharpened image shape: (540, 466)
Saved image to output.tif
```

TIFFReadDirectory: Warning, Unknown field with tag 34016 (0x84e0) encountered.

```
TIFFReadDirectory: Warning, Unknown field with tag 34017 (0x84e1) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34018 (0x84e2) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34019 (0x84e3) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34020 (0x84e4) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34021 (0x84e5) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34022 (0x84e6) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34023 (0x84e7) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34024 (0x84e8) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34025 (0x84e9) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34026 (0x84ea) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34027 (0x84eb) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34028 (0x84ec) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34029 (0x84ed) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34030 (0x84ee) encountered.
_TIFFVSetField: data/imgset3/Fig0338(a)(blurry_moon).tif: Null count for "Tag
34022" (type 1, writecount -3, passcount 1).
_TIFFVSetField: data/imgset3/Fig0338(a)(blurry_moon).tif: Null count for "Tag
34025" (type 1, writecount -3, passcount 1).
_TIFFVSetField: data/imgset3/Fig0338(a)(blurry_moon).tif: Null count for "Tag
34026" (type 1, writecount -3, passcount 1).
```



Laplacian sharpening (c=4)



Unsharp

[63]: | python python/main.py sharpen unsharp data/imgset3/Fig0338\(a\)\(blurry_moon\).

TIFFReadDirectory: Warning, Unknown field with tag 34016 (0x84e0) encountered. TIFFReadDirectory: Warning, Unknown field with tag 34017 (0x84e1) encountered.

```
TIFFReadDirectory: Warning, Unknown field with tag 34018 (0x84e2) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34019 (0x84e3) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34020 (0x84e4) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34021 (0x84e5) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34022 (0x84e6) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34023 (0x84e7) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34024 (0x84e8) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34025 (0x84e9) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34026 (0x84ea) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34027 (0x84eb) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34028 (0x84ec) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34029 (0x84ed) encountered.
TIFFReadDirectory: Warning, Unknown field with tag 34030 (0x84ee) encountered.
_TIFFVSetField: data/imgset3/Fig0338(a)(blurry_moon).tif: Null count for "Tag
34022" (type 1, writecount -3, passcount 1).
_TIFFVSetField: data/imgset3/Fig0338(a)(blurry_moon).tif: Null count for "Tag
34025" (type 1, writecount -3, passcount 1).
_TIFFVSetField: data/imgset3/Fig0338(a)(blurry_moon).tif: Null count for "Tag
34026" (type 1, writecount -3, passcount 1).
Sharpened image shape: (540, 466)
Saved image to output.tif
```

TIFFReadDirectory: Warning, Unknown field with tag 34016 (0x84e0) encountered. TIFFReadDirectory: Warning, Unknown field with tag 34017 (0x84e1) encountered. TIFFReadDirectory: Warning, Unknown field with tag 34018 (0x84e2) encountered. TIFFReadDirectory: Warning, Unknown field with tag 34019 (0x84e3) encountered. TIFFReadDirectory: Warning, Unknown field with tag 34020 (0x84e4) encountered. TIFFReadDirectory: Warning, Unknown field with tag 34021 (0x84e5) encountered. TIFFReadDirectory: Warning, Unknown field with tag 34022 (0x84e6) encountered. TIFFReadDirectory: Warning, Unknown field with tag 34023 (0x84e7) encountered. TIFFReadDirectory: Warning, Unknown field with tag 34024 (0x84e8) encountered. TIFFReadDirectory: Warning, Unknown field with tag 34025 (0x84e9) encountered. TIFFReadDirectory: Warning, Unknown field with tag 34026 (0x84ea) encountered. TIFFReadDirectory: Warning, Unknown field with tag 34027 (0x84eb) encountered. TIFFReadDirectory: Warning, Unknown field with tag 34028 (0x84ec) encountered. TIFFReadDirectory: Warning, Unknown field with tag 34029 (0x84ed) encountered. TIFFReadDirectory: Warning, Unknown field with tag 34030 (0x84ee) encountered. _TIFFVSetField: data/imgset3/Fig0338(a)(blurry_moon).tif: Null count for "Tag 34022" (type 1, writecount -3, passcount 1).

_TIFFVSetField: data/imgset3/Fig0338(a)(blurry_moon).tif: Null count for "Tag 34025" (type 1, writecount -3, passcount 1).
_TIFFVSetField: data/imgset3/Fig0338(a)(blurry_moon).tif: Null count for "Tag 34026" (type 1, writecount -3, passcount 1).





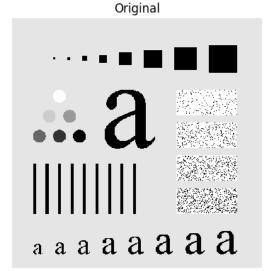
1.3.4 Magnitude of Gradient using Sobel Operators

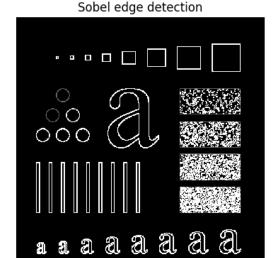
Implementation:

- Input: An 8-bit grayscale image.
- Process: Apply Sobel operators to the image to calculate the gradient in the x and y directions. Then, calculate the magnitude of the gradient at each pixel. Formula: $G = \sqrt{dx^2 + dy^2}$.
- Output: The blurred image.

```
[199]: | python python/main.py sobel data/imgset3/
-Fig0333\(a\)\(test_pattern_blurring_orig\).tif
```

Sobel image shape: (500, 500) Saved image to output.tif





1.3.5 Partial derivatives

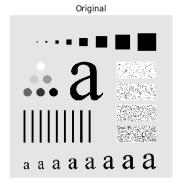
Implementation:

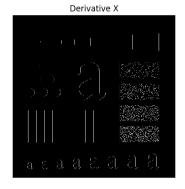
- Input: An 8-bit grayscale image.
- Process: Apply partial derivative convolution kernels to the image to calculate the gradients in the x and y directions.
- Output: The blurred image.

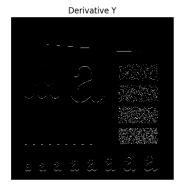
```
[202]: !python python/main.py apply-kernel derivative_x data/imgset3/
    Fig0333\(a\)\(test_pattern_blurring_orig\).tif --output-path output_x.tif
!python python/main.py apply-kernel derivative_y data/imgset3/
    Fig0333\(a\)\(test_pattern_blurring_orig\).tif --output-path output_y.tif
!python python/main.py apply-kernel second_derivative_x data/imgset3/
    Fig0333\(a\)\(test_pattern_blurring_orig\).tif --output-path output_x2.tif
!python python/main.py apply-kernel second_derivative_y data/imgset3/
    Fig0333\(a\)\(test_pattern_blurring_orig\).tif --output-path output_y2.tif
```

```
Transformed image shape: (500, 500)
Saved image to output_x.tif
Transformed image shape: (500, 500)
Saved image to output_y.tif
Transformed image shape: (500, 500)
Saved image to output_x2.tif
Transformed image shape: (500, 500)
Saved image to output_y2.tif
```

First derivatives







Second derivatives

