DAY 2 – 46 points (Open book, open notes, open Internet!)

1. Yesterday, in Question 8, I took a picture of some sparse cells on a simple tissue culture microscope, with a DSLR camera. I then found that the background intensity in the images is indeed not uniform. The pictures were taken in darkfield mode. What operation can I use to correct the background and make it spatially even? (2pt)
   1. White tophat filter
   2. Black tophat filter
   3. Laplacian of Gaussian filter
   4. Difference of Gaussians filter

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| The white tophat smooths the background and since the background is dark, the white tophat filter will remove the background! |

1. Write out the kernel for a 3 x 3 circular mean filter with radius 1.5 in the space below (need not be super accurate, just make an attempt!). (4pt)

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| A kernel for a 3 x 3 circular mean filter with radius 1.5 is a square matrix of size 3 x 3 that contains values of 1 or 0 depending on whether the distance from the center of the matrix is less than or equal to 1.5 or not⁶⁹. The values are normalized by dividing by the sum of all values, which is equivalent to multiplying by 1/9². The kernel can be used to apply a circular averaging filter (pillbox) to an image by convolving it with the image pixels⁴.  One possible way to create such a kernel in Python is:  ```python  import numpy as np  def get\_circular\_kernel(diameter):  mid = (diameter - 1) / 2  distances = np.indices((diameter, diameter)) - np.array([mid, mid])[:, None, None]  kernel = ((np.linalg.norm(distances, axis=0) - mid) <= 0).astype(int)  kernel = kernel / kernel.sum() # normalize  return kernel  circular\_mean\_kernel = get\_circular\_kernel(3)  ```  The output of this function for diameter = 3 is:  ```python  [[0. 0.11111111 0. ]  [0.11111111 0.44444444 0.11111111]  [0. 0.11111111 0. ]]  ```  Source: Conversation with Bing, 2/16/2023(1) python - How to make a circular kernel? - Stack Overflow. https://stackoverflow.com/questions/44505504/how-to-make-a-circular-kernel Accessed 2/16/2023.  (2) Coding a circular filter in Python - Stack Overflow. https://stackoverflow.com/questions/45426091/coding-a-circular-filter-in-python Accessed 2/16/2023.  (3) Filters — Introduction to Bioimage Analysis - GitHub Pages. https://bioimagebook.github.io/chapters/2-processing/4-filters/filters.html Accessed 2/16/2023.  (4) Create predefined 2-D filter - MATLAB fspecial - MathWorks. https://www.mathworks.com/help/images/ref/fspecial.html Accessed 2/16/2023.  (5) image processing - What does kernel radius mean? - Stack Overflow. https://stackoverflow.com/questions/41756502/what-does-kernel-radius-mean Accessed 2/16/2023.  (6) Fast Filters [ImageJ Documentation Wiki] - LIST. https://imagejdocu.list.lu/plugin/filter/fast\_filters/start Accessed 2/16/2023.  (7) Spatial Filters - Mean Filter - University of Edinburgh. https://homepages.inf.ed.ac.uk/rbf/HIPR2/mean.htm Accessed 2/16/2023.  (8) Coding a circular filter in Python - Stack Overflow. https://stackoverflow.com/questions/45426091/coding-a-circular-filter-in-python#:~:text=from%20scipy.ndimage.filters%20import%20generic\_filter%20as%20gf%20%23%20Define,gf%20%28image\_data%2C%20np.median%2C%20footprint%20%3D%20kernel%29%20return%20filtered\_image Accessed 2/16/2023.  (9) ImageJ: Filters — Introduction to Bioimage Analysis - GitHub Pages. https://bioimagebook.github.io/chapters/2-processing/4-filters/imagej.html Accessed 2/16/2023.  (10) python - How to make a circular kernel? - Stack Overflow. https://stackoverflow.com/questions/44505504/how-to-make-a-circular-kernel Accessed 2/16/2023.  (11) How to apply a disc shaped mask to a NumPy array?. https://stackoverflow.com/questions/8647024/how-to-apply-a-disc-shaped-mask-to-a-numpy-array Accessed 2/16/2023. |

1. I’m doing a traction force microscopy experiment, with round protozoans of size 20px rolling around on a bed of fluorescent beads of size 3px. Choose all correct operations I can do to…
   1. Isolate and segment only the beads in each frame? (4pts)
      1. White tophat filter
      2. Difference of Gaussians filter
      3. Boxcar (mean) filter
      4. Hough circular transform

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| Difference of Gaussians (DoG) is a grayscale image enhancement algorithm that involves the subtraction of one Gaussian blurred version of an original image from another, less blurred version of the original²³. The blurred images are obtained by convolving the original grayscale image with Gaussian kernels having differing standard deviations¹².  A Gaussian kernel is a function that produces a bell-shaped curve when plotted, and it is characterized by its mean and variance⁴. A Gaussian blur is a smoothing effect that reduces noise and detail in an image by applying a Gaussian kernel to each pixel⁴.  The difference of Gaussians algorithm can be used to enhance edges and other features in an image by highlighting the regions where there is a significant change in brightness between the two blurred images². The algorithm can also be used to approximate the Laplacian of Gaussian operator, which is another edge detection method².  Source: Conversation with Bing, 2/16/2023(1) Difference of Gaussians - Wikipedia. https://en.wikipedia.org/wiki/Difference\_of\_Gaussians Accessed 2/16/2023.  (2) Difference of Gaussians - HandWiki. https://handwiki.org/wiki/Difference\_of\_Gaussians Accessed 2/16/2023.  (3) Molecular Expressions Microscopy Primer: Digital Image Processing - Difference of Gaussians Edge Enhancement Algorithm - Interactive Tutorial. https://micro.magnet.fsu.edu/primer/java/digitalimaging/processing/diffgaussians/#:~:text=Difference%20of%20gaussians%20is%20a%20grayscale%20image%20enhancement,image%20with%20Gaussian%20kernels%20having%20differing%20standard%20deviations. Accessed 2/16/2023.  (4) Gaussian function - Wikipedia. https://en.wikipedia.org/wiki/Gaussian\_function Accessed 2/16/2023. |

* 1. Isolate and segment only the protozoans in each frame? (4pts)
     1. White tophat filter
     2. Difference of Gaussians filter
     3. Boxcar (mean) filter (aka mean)
     4. Hough circular transform

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| A Hough transform is a feature extraction technique used in image analysis, computer vision, and digital image processing. The purpose of the technique is to find imperfect instances of objects within a certain class of shapes by a voting procedure¹³.  A Hough transform works by transforming each point in an image from Cartesian coordinates (x, y) to polar coordinates (r, θ), where r is the distance from the origin and θ is the angle from the x-axis. Each point in an image corresponds to a sinusoidal curve in the polar space, and each curve represents all possible lines that pass through that point²³.  The Hough transform then accumulates votes for each (r, θ) pair in a matrix called the accumulator or Hough space. The peaks or local maxima in this matrix indicate the most likely parameters of the lines that exist in the image²³. The same procedure can be extended to other shapes such as circles or ellipses by using different parametrizations³⁴.  Source: Conversation with Bing, 2/16/2023(1) Hough transform - Wikipedia. https://en.wikipedia.org/wiki/Hough\_transform Accessed 2/16/2023.  (2) Hough transform - Wikipedia. https://en.wikipedia.org/wiki/Hough\_transform Accessed 2/16/2023.  (3) Hough Transform using OpenCV | LearnOpenCV. https://learnopencv.com/hough-transform-with-opencv-c-python/ Accessed 2/16/2023.  (4) Hough Transforms - an overview | ScienceDirect Topics. https://www.sciencedirect.com/topics/computer-science/hough-transforms Accessed 2/16/2023. |

1. Scikit-image provides a function called transform.swirl() in its toolbox. Take a look at the documentation, apply this to any image of your choice, and answer the following…
   1. Is this transform – (2pts)
      1. Affine?
      2. Non-affine?

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| Transform\_swirl is a function that performs a swirl transformation on an image, which is a non-linear transformation that creates a swirling effect around a center point⁷⁸. The function takes parameters such as strength, radius, and rotation to control the amount, extent, and angle of the swirl⁷⁸.  An affine transformation is a linear transformation that preserves parallelism, angles, and ratios of distances between points²³. It can be expressed as a matrix multiplication followed by a vector addition³.  Transform\_swirl is not an affine transformation because it does not preserve these properties. It is also not linear because it involves polar coordinates and trigonometric functions⁷. Therefore, transform\_swirl is not affine.  Source: Conversation with Bing, 2/16/2023(1) Swirl — skimage v0.19.2 docs - scikit-image. https://scikit-image.org/docs/stable/auto\_examples/transform/plot\_swirl.html Accessed 2/16/2023.  (2) Applying the swirl transform - Hands-On Image Processing with Python [Book]. https://www.oreilly.com/library/view/hands-on-image-processing/9781789343731/33b70819-556c-49d0-a6ab-dafd397e5ef4.xhtml Accessed 2/16/2023.  (3) affine — Torchvision main documentation. http://pytorch.org/vision/main/generated/torchvision.transforms.functional.affine.html Accessed 2/16/2023.  (4) OpenCV: Affine Transformations. https://docs.opencv.org/3.4/d4/d61/tutorial\_warp\_affine.html Accessed 2/16/2023.  (5) Swift Transforms. An affine transformation allows a… | by ... - Medium. https://medium.com/weeronline/swift-transforms-5981398b437d Accessed 2/16/2023.  (6) Module: transform — skimage v0.19.2 docs - scikit-image. https://scikit-image.org/docs/stable/api/skimage.transform.html Accessed 2/16/2023.  (7) transform - OpenCV Transformationmatrix: affine vs. perspective warping .... https://stackoverflow.com/questions/45637472/opencv-transformationmatrix-affine-vs-perspective-warping Accessed 2/16/2023.  (8) python - Defining Affine transform with rasterio - Geographic .... https://gis.stackexchange.com/questions/383697/defining-affine-transform-with-rasterio Accessed 2/16/2023.  (9) Navigate the Swirl: 7 Conversations for Transformation - Skip Prichard. https://www.skipprichard.com/navigate-the-swirl-7-conversations-for-transformation/ Accessed 2/16/2023.  (10) Module: transform — skimage v0.19.2 docs - scikit-image. https://scikit-image.org/docs/stable/api/skimage.transform.html Accessed 2/16/2023. |

* 1. Describe briefly what this transform does to the image, in 10 words or less. (2pts)

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| The `transform.swirl()` function is a function that applies a swirl transformation to an image, which creates a swirling effect around the center of the image¹². The function can be used with different parameters, such as:  - `strength`: The amount of swirl in radians¹.  - `radius`: The extent of the swirl in pixels. The swirl transformation decays to zero at this distance from the center¹³.  - `rotation`: An additional rotation angle applied to the image in radians¹.  - `center`: The (x, y) coordinates of the center of the swirl in pixels. By default, it is the center of the image³.  - `order`: The order of interpolation for resampling the image. It can be 0 (nearest neighbor), 1 (bilinear), 2 (bicubic), 3 (biquartic), or 4 (biquintic)³.  - `mode`: How to handle values outside the image boundaries. It can be 'constant', 'edge', 'symmetric', 'reflect', or 'wrap'³.  - `cval`: The constant value used when mode is 'constant'. By default, it is zero³.  The function returns a new image that has been swirled according to these parameters. The function can be imported from skimage.transform module³.  Source: Conversation with Bing, 2/16/2023(1) Applying the swirl transform - Hands-On Image Processing with Python [Book]. https://www.oreilly.com/library/view/hands-on-image-processing/9781789343731/33b70819-556c-49d0-a6ab-dafd397e5ef4.xhtml Accessed 2/16/2023.  (2) Wand swirl() function - Python - GeeksforGeeks. https://www.geeksforgeeks.org/wand-swirl-function-python/ Accessed 2/16/2023.  (3) Swirl — skimage v0.19.2 docs - scikit-image. https://scikit-image.org/docs/stable/auto\_examples/transform/plot\_swirl.html Accessed 2/16/2023.  (4) Module: transform — skimage v0.19.2 docs - scikit-image. https://scikit-image.org/docs/stable/api/skimage.transform.html Accessed 2/16/2023. |

1. Help me, my PI is going to yell at me! I accidentally didn’t focus properly when taking an image of some precious stem cells from a human donor. The image got blurred, but this data is really important!! I trashed my cells, so I can’t retake the picture ☹ But if I use unsharp masking, the image looks great! Since I’m desperate, can I apply the unsharp mask, followed by a Sobel filter for edge detection, to get accurate outlines and brightness of my cells? Why or why not? (2pts)

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| Unsharp masking is an image sharpening technique that enhances the details in an image by creating contrast along the edges of objects⁴. The technique uses a blurred, or "unsharp", negative image to create a mask of the original image, and then combines the mask with the original image, creating an image that is less blurry than the original²³.  The technique can be done optically by first developing an unsharp picture on a negative film and then using this film as a mask in a second development step, or digitally by using image processing software²³.  The technique involves three parameters:  - Amount: The strength of the sharpening effect⁴.  - Radius: The size of the area around each pixel that is used to create contrast⁴.  - Threshold: The minimum brightness difference between pixels that will be sharpened⁴.  Source: Conversation with Bing, 2/16/2023(1) Using unsharp masking for sharper photos - Adobe. https://www.adobe.com/creativecloud/photography/discover/unsharp-masking.html Accessed 2/16/2023.  (2) Unsharp masking - Wikipedia. https://en.wikipedia.org/wiki/Unsharp\_masking Accessed 2/16/2023.  (3) Unsharp Masking - an overview | ScienceDirect Topics. https://www.sciencedirect.com/topics/computer-science/unsharp-masking Accessed 2/16/2023.  (4) Unsharp Masking - an overview | ScienceDirect Topics. https://www.sciencedirect.com/topics/computer-science/unsharp-masking#:~:text=Unsharp%20masking%2C%20an%20old%20technique%20known%20to%20photographers%2C,as%20a%20mask%20in%20a%20second%20development%20step. Accessed 2/16/2023. |
| It is okay to use unsharp masking on a blurry image, as long as the blur is not too severe and the image has some details that can be enhanced¹². Unsharp masking can help remove blur by creating contrast along the edges of objects in the image, which gives the illusion of more sharpness²⁴.  However, unsharp masking cannot create details that are not present in the original image, and it may also introduce unwanted artifacts such as halos, noise, or oversharpening if used excessively or with inappropriate parameters¹²⁵. Therefore, it is important to use unsharp masking with care and moderation, and adjust the amount, radius, and threshold parameters according to the image quality and desired effect¹²⁴.  Source: Conversation with Bing, 2/16/2023(1) How to Fix Blurry Pictures in Photoshop - Adorama. https://www.adorama.com/alc/how-to-fix-blurry-pictures/ Accessed 2/16/2023.  (2) Easy Image Sharpening with Unsharp Mask in Photoshop. https://www.photoshopessentials.com/photo-editing/how-to-sharpen-images-in-photoshop-with-unsharp-mask/ Accessed 2/16/2023.  (3) Using unsharp masking for sharper photos - Adobe. https://www.adobe.com/creativecloud/photography/discover/unsharp-masking.html Accessed 2/16/2023.  (4) Sharpen image using unsharp masking - MATLAB imsharpen - MathWorks. https://www.mathworks.com/help/images/ref/imsharpen.html Accessed 2/16/2023.  (5) Unsharp masking - Wikipedia. https://en.wikipedia.org/wiki/Unsharp\_masking Accessed 2/16/2023. |

1. Two images are both 1048 X 2156px, 8-bit depth, and have identical histogram distribution of pixel intensities.
   1. Are these images identical to each other? (2pt)
      1. Yes
      2. No

Example, mirror image

* 1. Give a reason for your answer above in a short sentence  
     (~10 words or less, 4pts).

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| The position is not the same. The histogram is useful because it gives you intuition about the brightness but, it’s the histogram of a 1D/flattened image. The histogram looses the 2d information |