Lecture 16: Introduction to Physical Layers in Machine Learning

Machine Learning and Imaging

BME 548L
Roarke Horstmeyer

deep imaging

Notes

- Homework 3 due Monday 3/25 at 11pm
- Homework 4 posted most likely next Monday, due 2 weeks later

- I'll review final project proposals this week

Project proposal review meetings

I would like to have a short (15 min.) 1-on-1 meeting with all final project teams this week to provide feedback on your project proposals. It is also an opportunity for you all to ask me questions, and to refine your strategy for your final project as needed early on, to maximize chances for success

Please sign up for a 15 minute time to chat here:

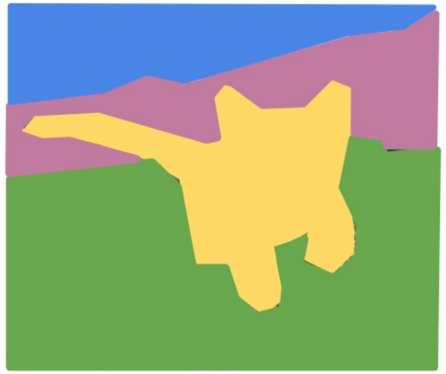
https://calendly.com/rwh4/15min

We can use the course zoom link for the meetings:

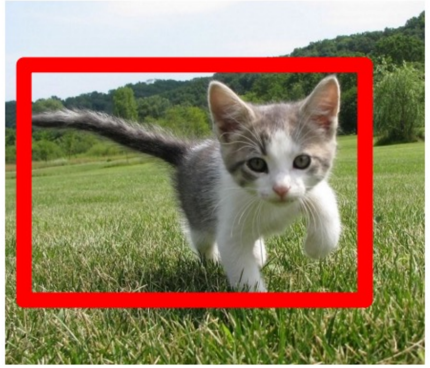
https://duke.zoom.us/j/93342721843

Other Computer Vision Tasks

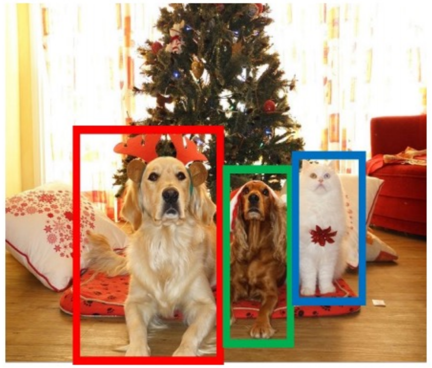
Semantic Segmentation | **Classification + Localization** | **Object Detection** | **Instance Segmentation** | **Super-resolution**



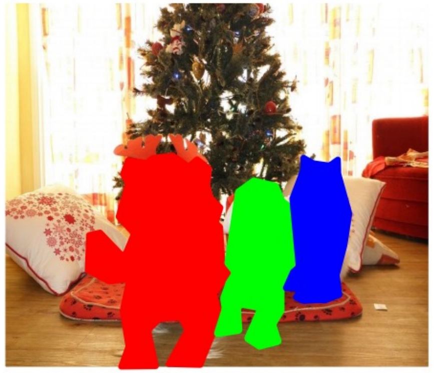
GRASS, CAT, TREE, SKY



CAT



DOG, DOG, CAT



DOG, DOG, CAT

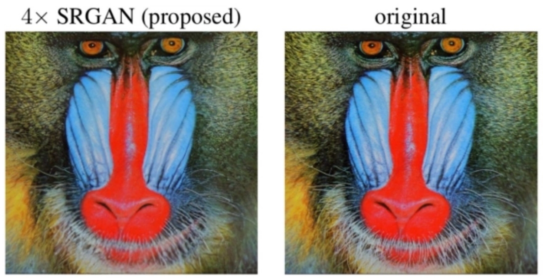


Figure 1: Super-resolved image (left) is almost indistinguishable from original (right). [4× upscaling]

No objects, just pixels

Single Object

Multiple Object

This image is CC0 public domain

Balanced equation

Over-determined

Over-determined

Over-determined

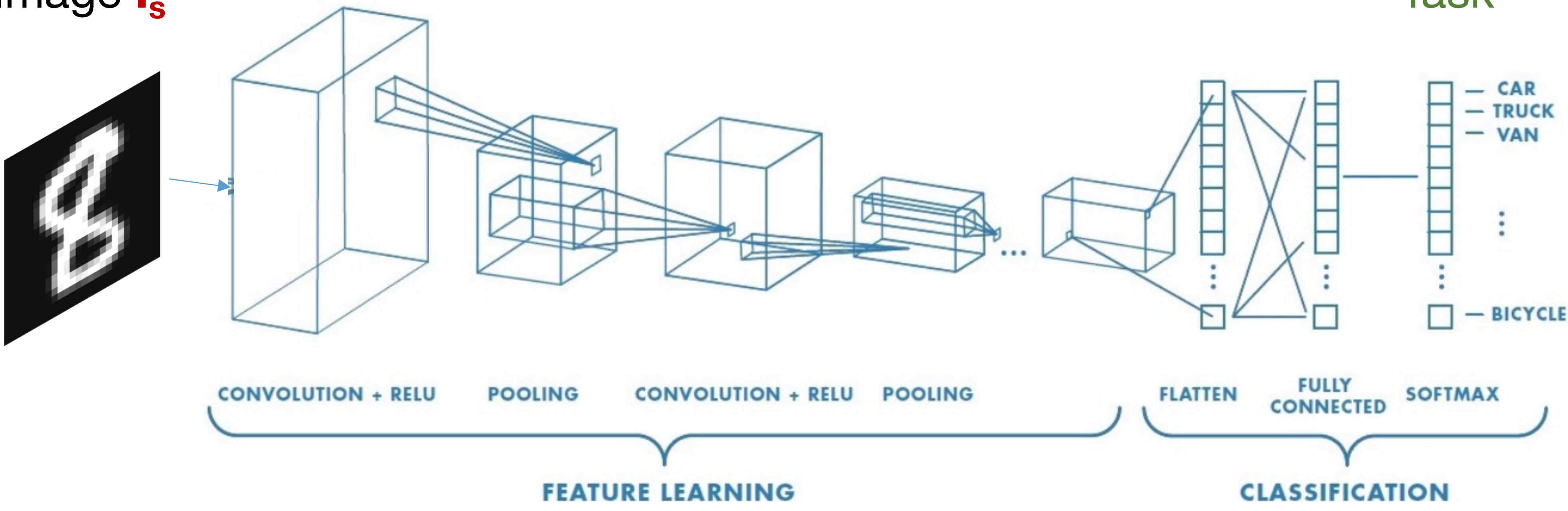
Under-determined

Stanford CS231n - http://cs231n.stanford.edu

Bringing together physical and digital image representations

Image $\mathbf{I_s}$

Task



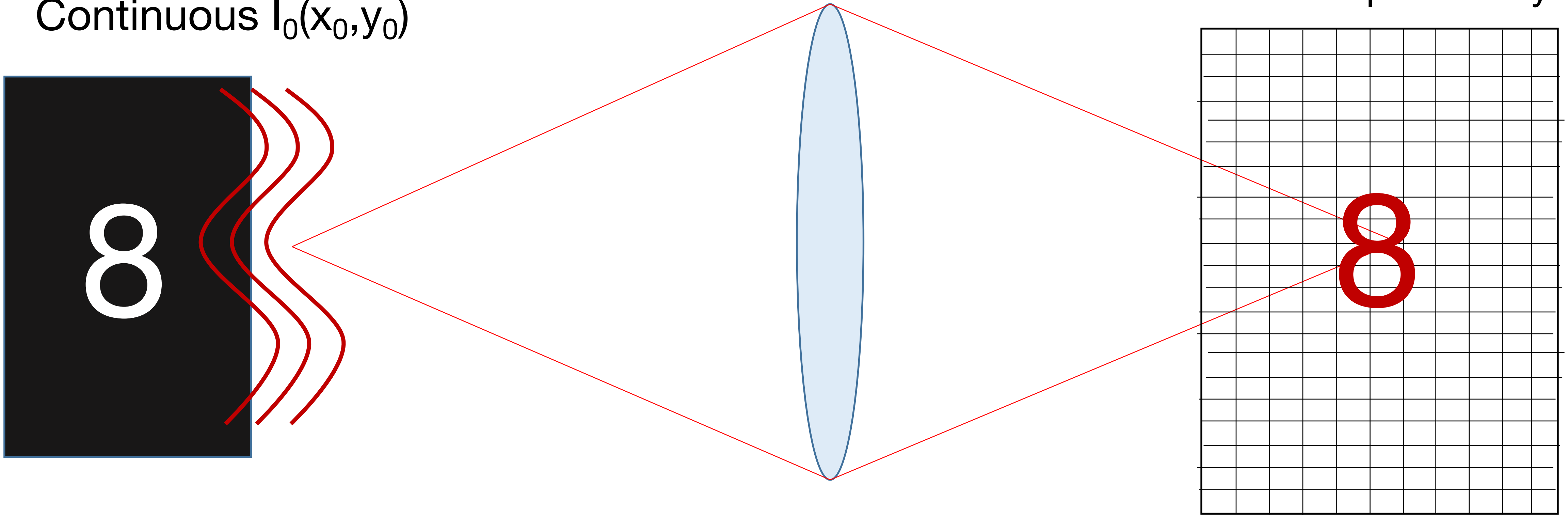
$$\text{Task} = \mathbf{W_n} \ldots \text{ReLU}[\mathbf{W_1}\, \text{ReLU}[\mathbf{W_0}\, \mathbf{I_s}]\ldots]$$

deep imaging

Simple model of image formation

Continuous $I_0(x_0,y_0)$

n x *m* pixel array



Discrete signal

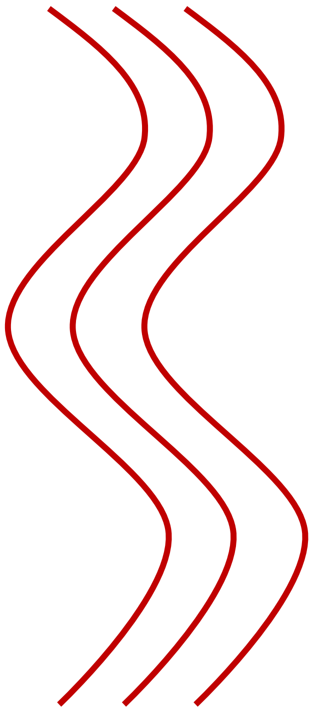
$$\boxed{I_s(x,y), (x,y) \in Z^{n\times m}}$$

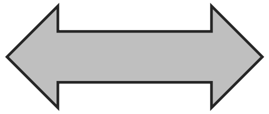
Digitazation

Photons to electrons

What does the Sampling Theorem mean for us?

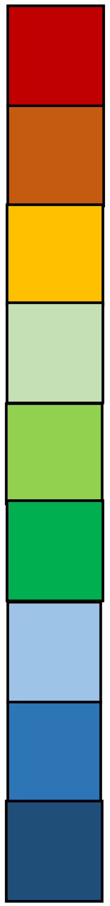
Continuous functions





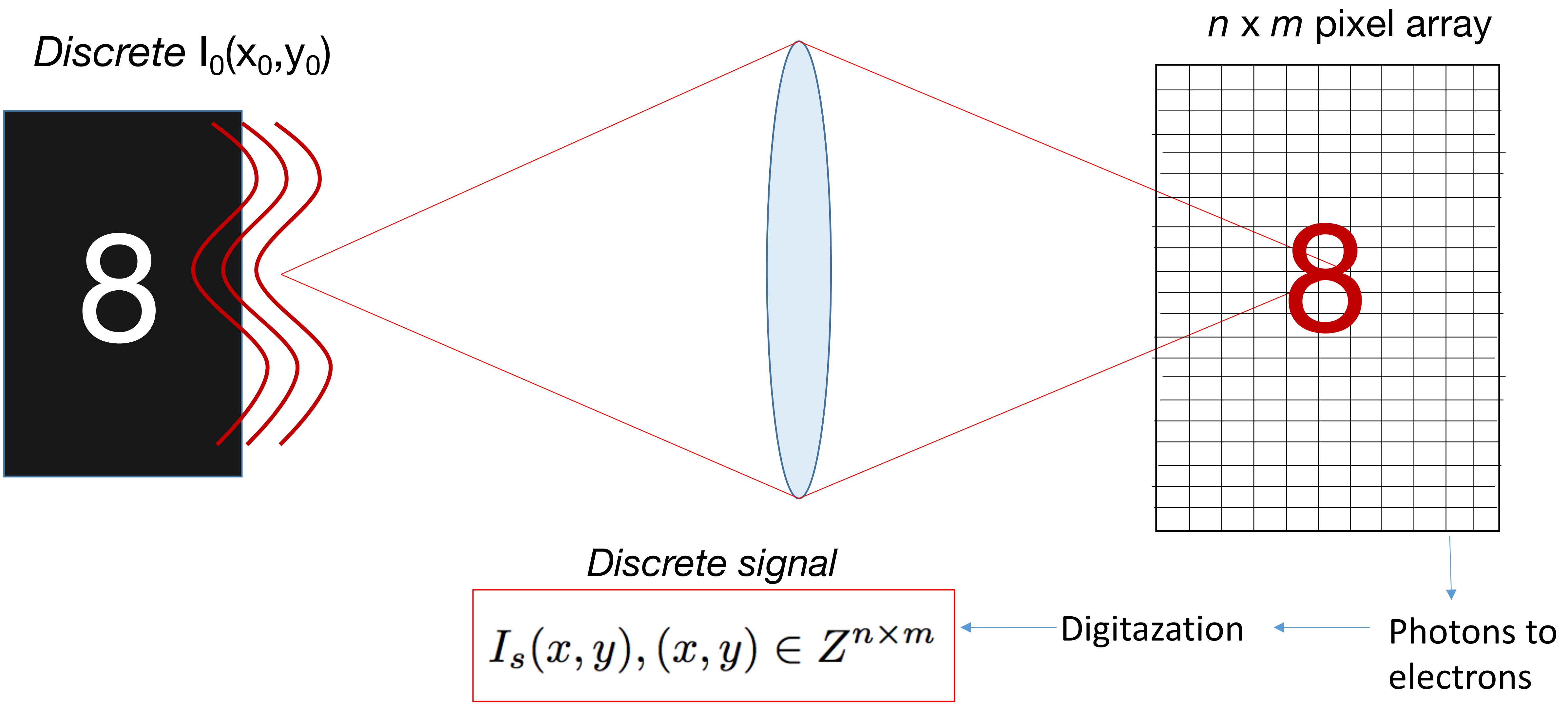
*conditions

Discretize vectors
(and matrices)



17
20
22
21
23
25
24
26
29

Simple model of image formation



Bringing together physical and digital image representations

deep imaging

Physical Layers

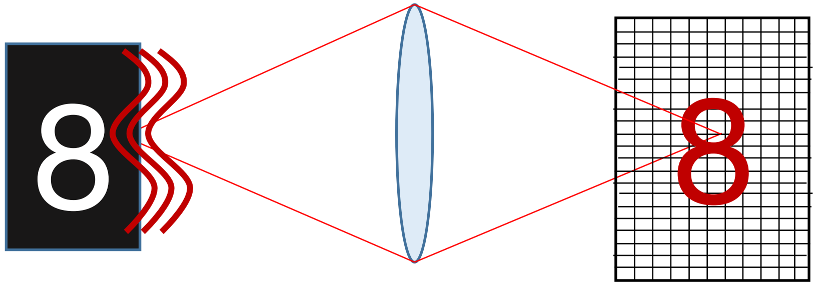
Digital Layers

Physical world

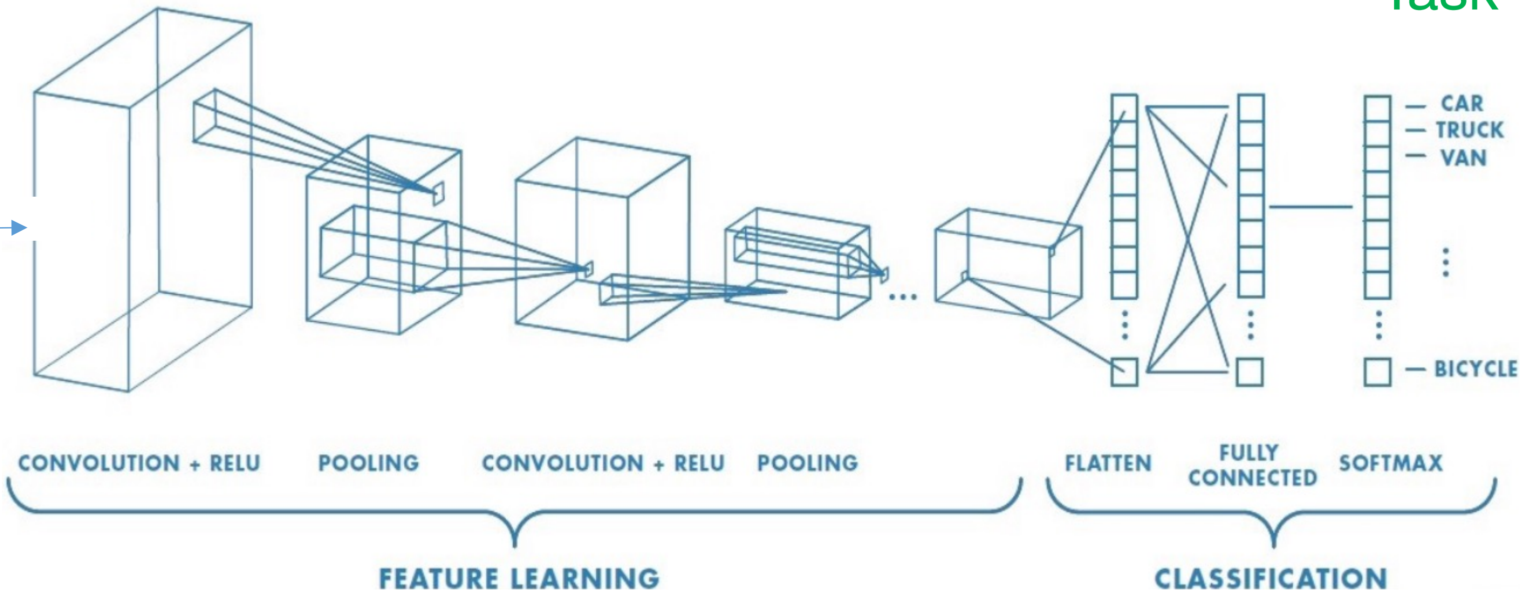
$\mathbf{I_0}(x_0,y_0)$

Digital Image

$\mathbf{I_s}(x,y)$



Task



$$\mathbf{I_s} = f[\mathbf{I_0}]$$

Digital layers

Physical layers

$$\text{Task} = \mathbf{W_n} \ldots \mathrm{ReLU}[\mathbf{W_1}\ \mathrm{ReLU}[\mathbf{W_0}\ f[\mathbf{I_0}]]\ldots]$$

Bringing together physical and digital image representations

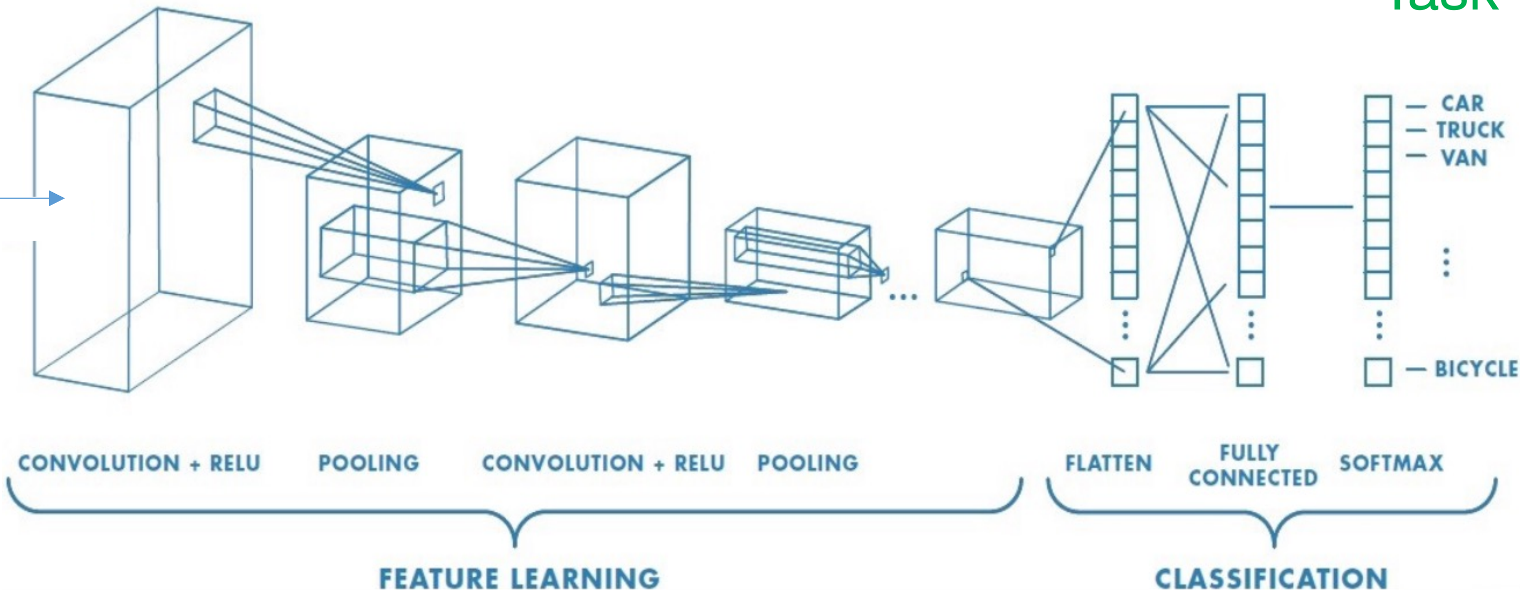
deep imaging

Physical Layers

Digital Layers

Task

Physical Function $\mathbf{I_0}$ → f[ ] → Digitized $\mathbf{I_s}$ →



$$\mathbf{I_s} = f[\mathbf{I_0}]$$

Digital layers

Physical layers

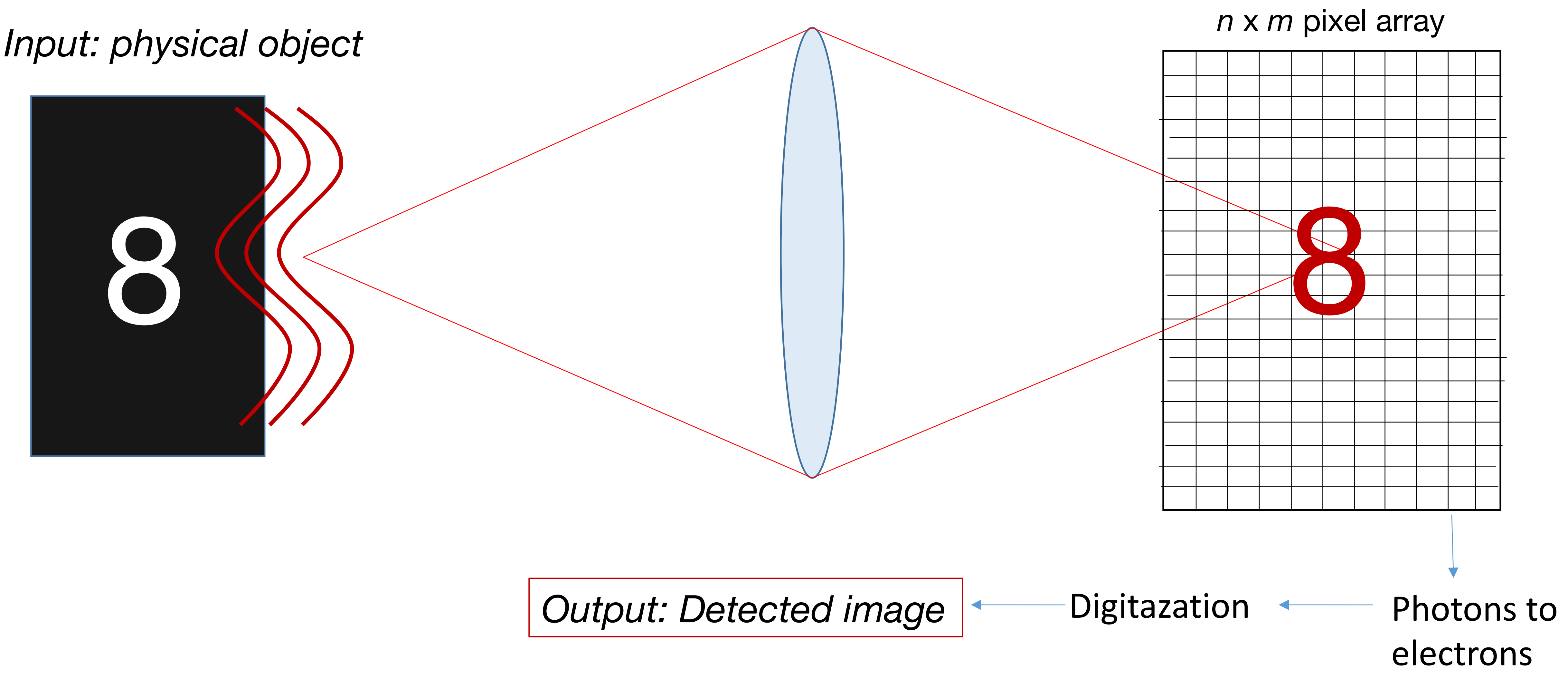
$$\text{Task} = \mathbf{W}_n \ldots \mathrm{ReLU}[\mathbf{W}_1\, \mathrm{ReLU}[\mathbf{W}_0\, f[\mathbf{I_0}]] \ldots]$$

Required properties of physical mapping f[ ] for DNN optimization?

- Finite
- Non-zero gradients
- Differentiable*
- Known structure (for now…)
- Anything else?

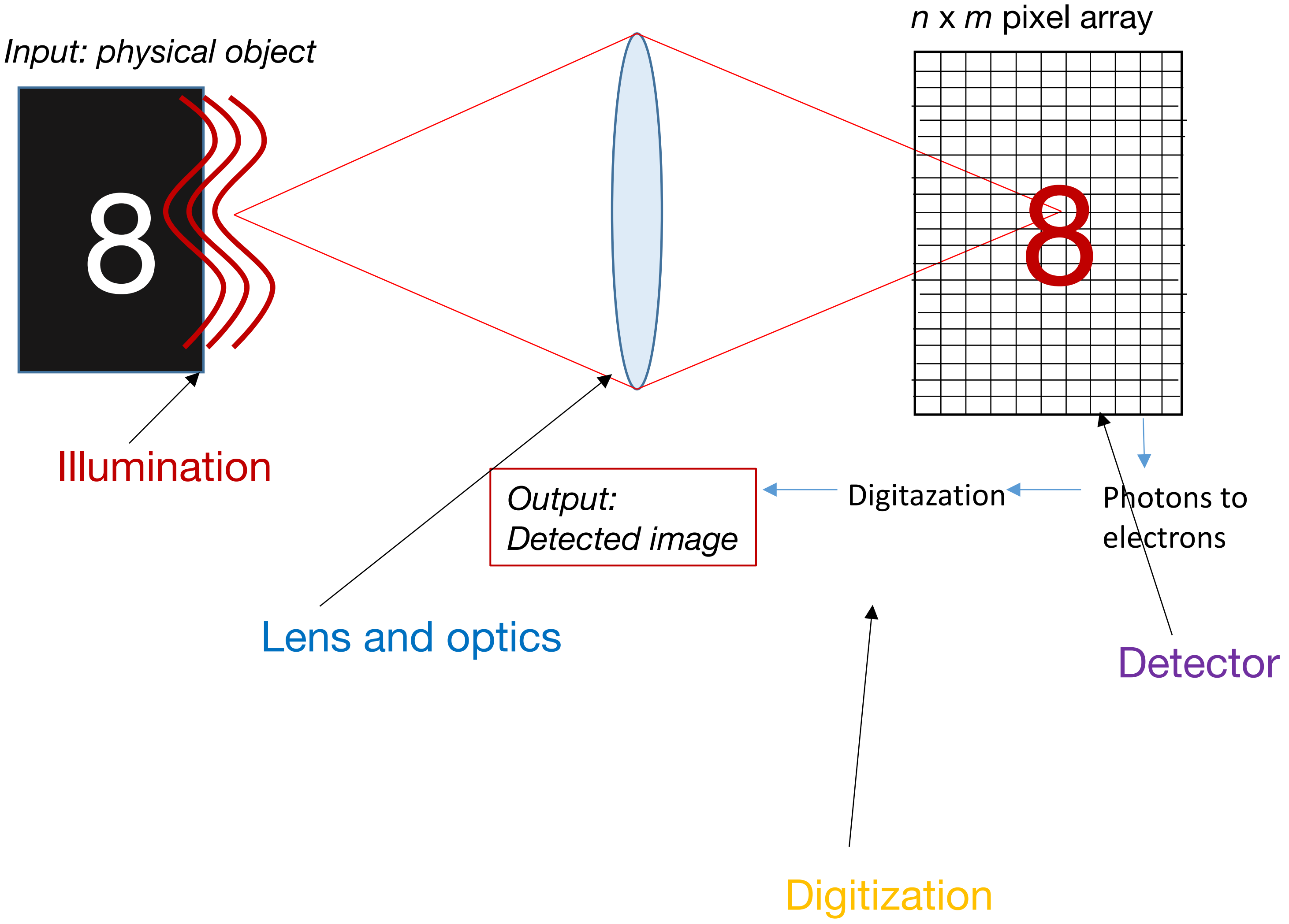
What physical parameters effect image formation?

deep imaging

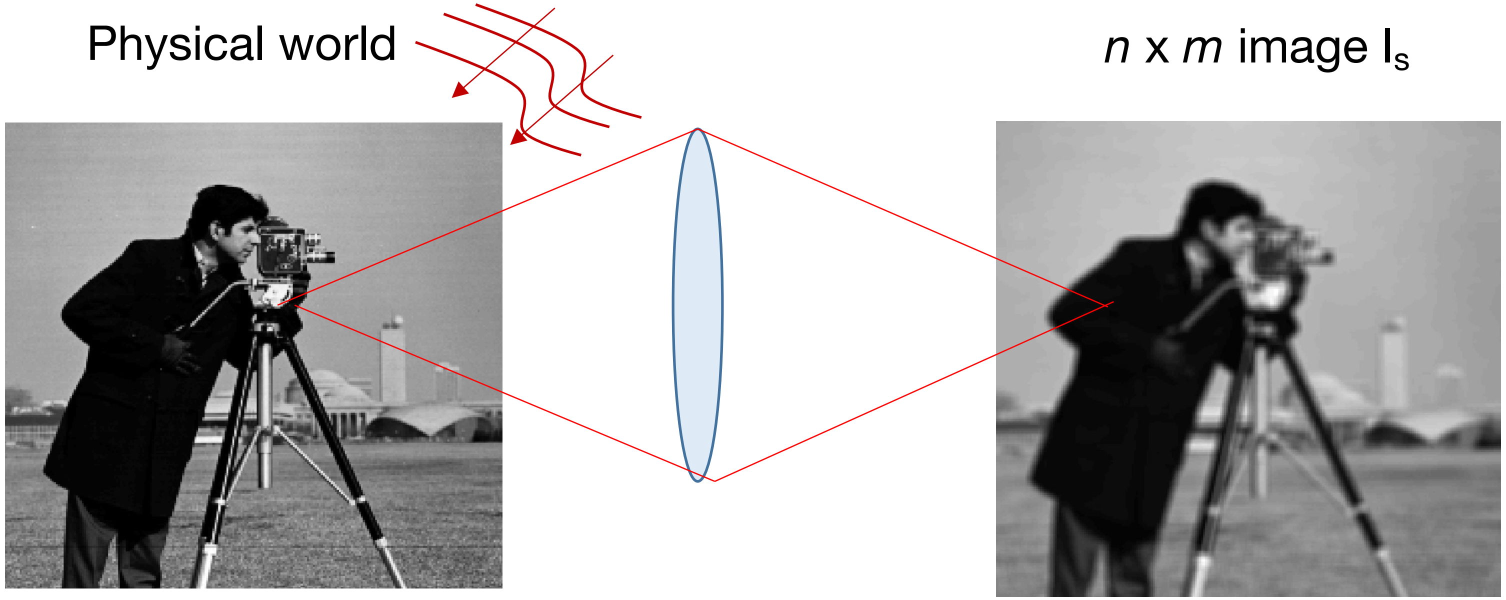


What physical parameters effect image formation?

- Illumination
  - Spatial pattern
  - Angle of incidence
  - Color, polarization
- Lens and optics
  - Position/orientation
  - Shape
  - Focus
  - Transparency
- Detector
  - Pixel size
  - Pixel shape & fill factor
  - Color filters
  - Other filters
- Digitization
  - E to P curves
  - Digitization schemes/thresholds
  - Data transmission, multiplexing
- Physical object

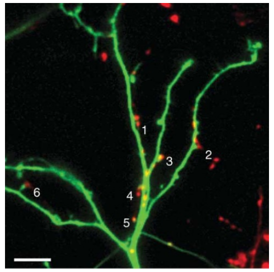


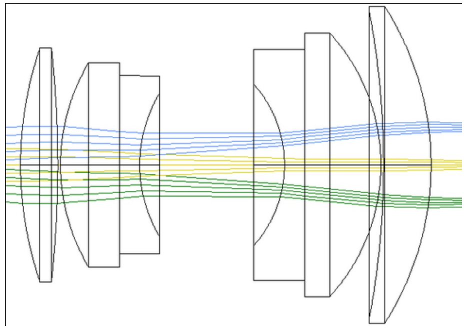
Simple mathematical model of image formation



First - what is light and how can we model it?

- Interpretation #1: Radiation (*Incoherent*)
- Model: Rays





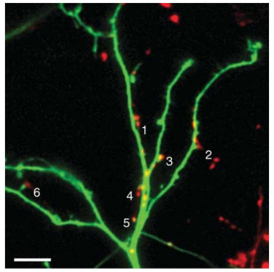
- Real, non-negative
- Models absorption and brightness

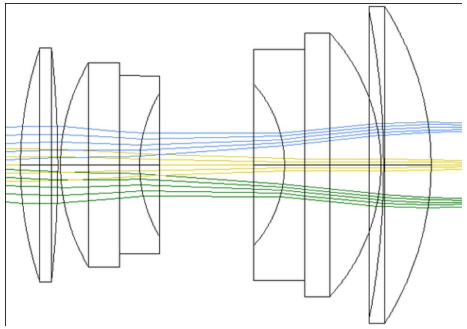
$$I_{tot} = I_1 + I_2$$

First - what is light and how can we model it?

deep imaging

- Interpretation #1: Radiation (*Incoherent*)
- Model: Rays

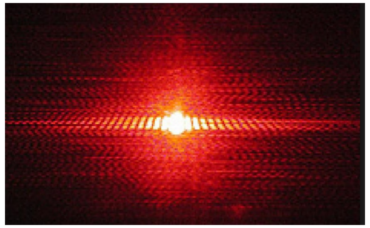


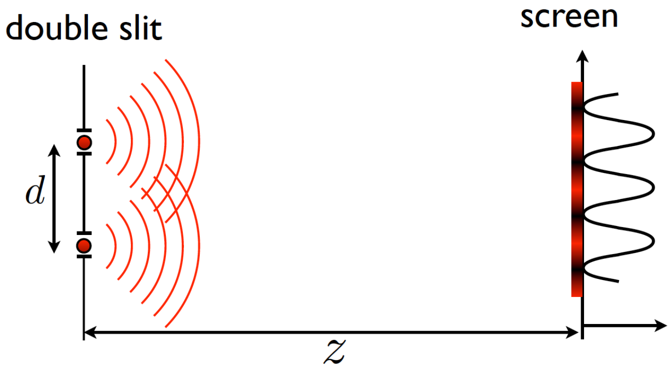


- Real, non-negative
- Models absorption and brightness

$I_{tot} = I_1 + I_2$

- Interpretation #2: Electromagnetic wave (*Coherent*)
- Model: Waves



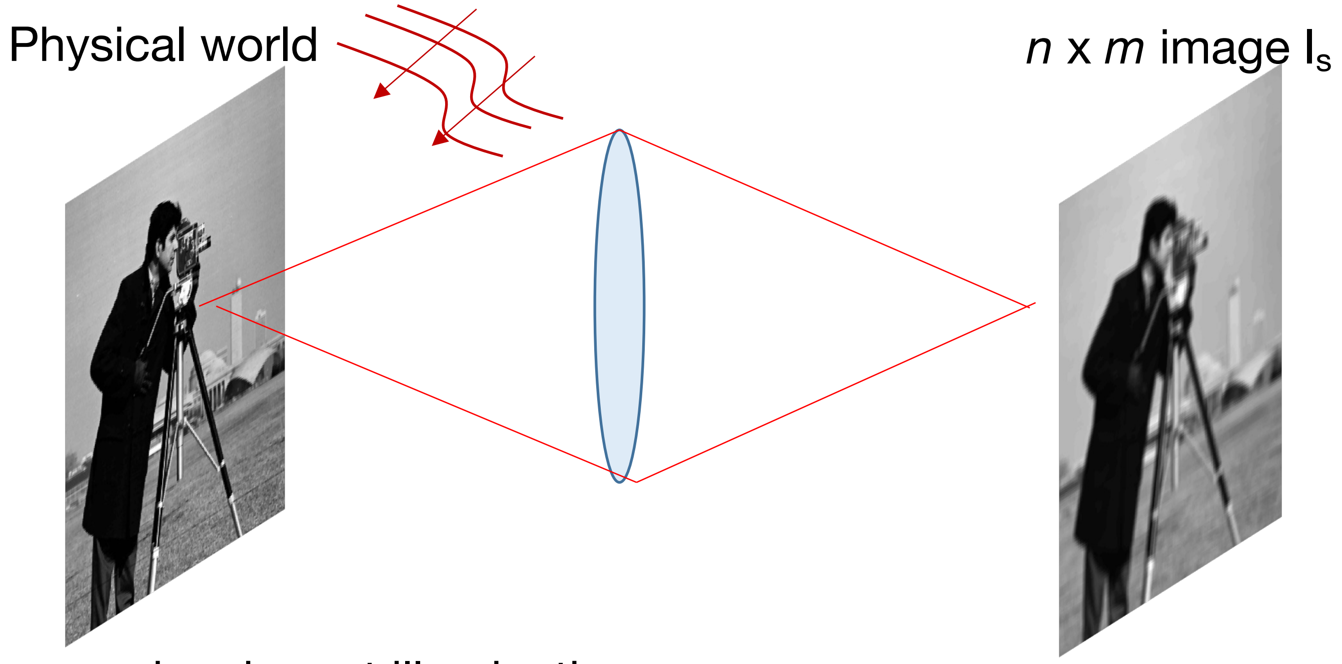


- Complex field
- Models Interference

$E_{tot} = E_1 + E_2$

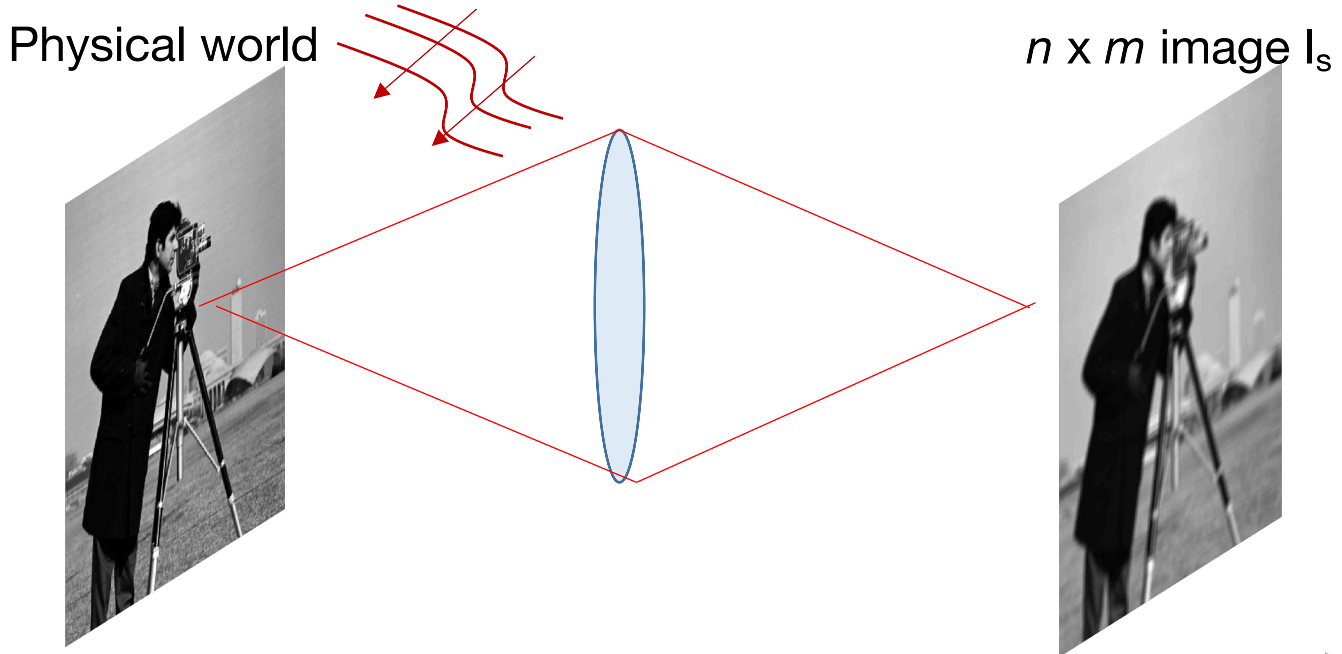
- Interpretation #3: Particle
- Model: Photons

Simple mathematical model of incoherent image formation



- Assume incoherent illumination
- Assume thin 2D object
- Object is real, non-negative map of absorption/reflectivity

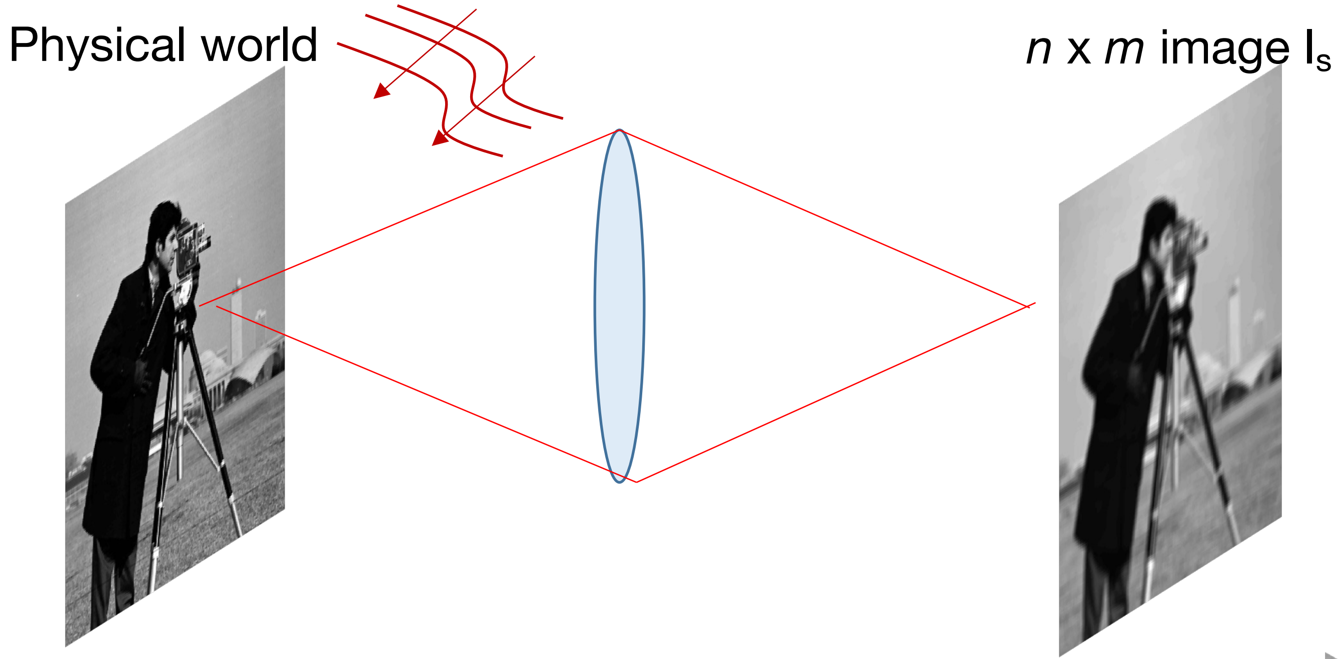
Simple mathematical model of incoherent image formation



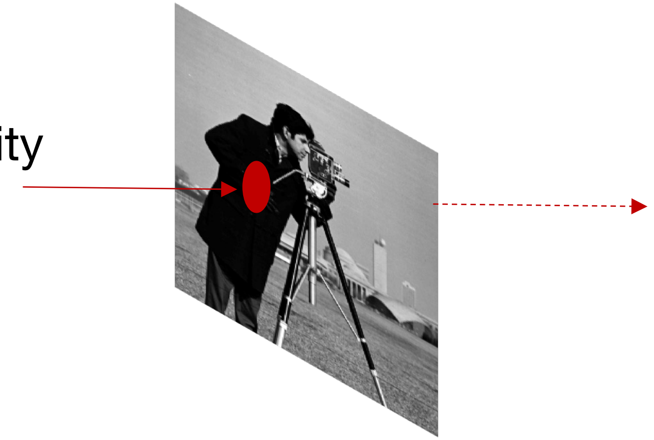
- Assume incoherent illumination
- Assume thin 2D object
- Object is real, non-negative map of absorption/reflectivity

Simple mathematical model of incoherent image formation

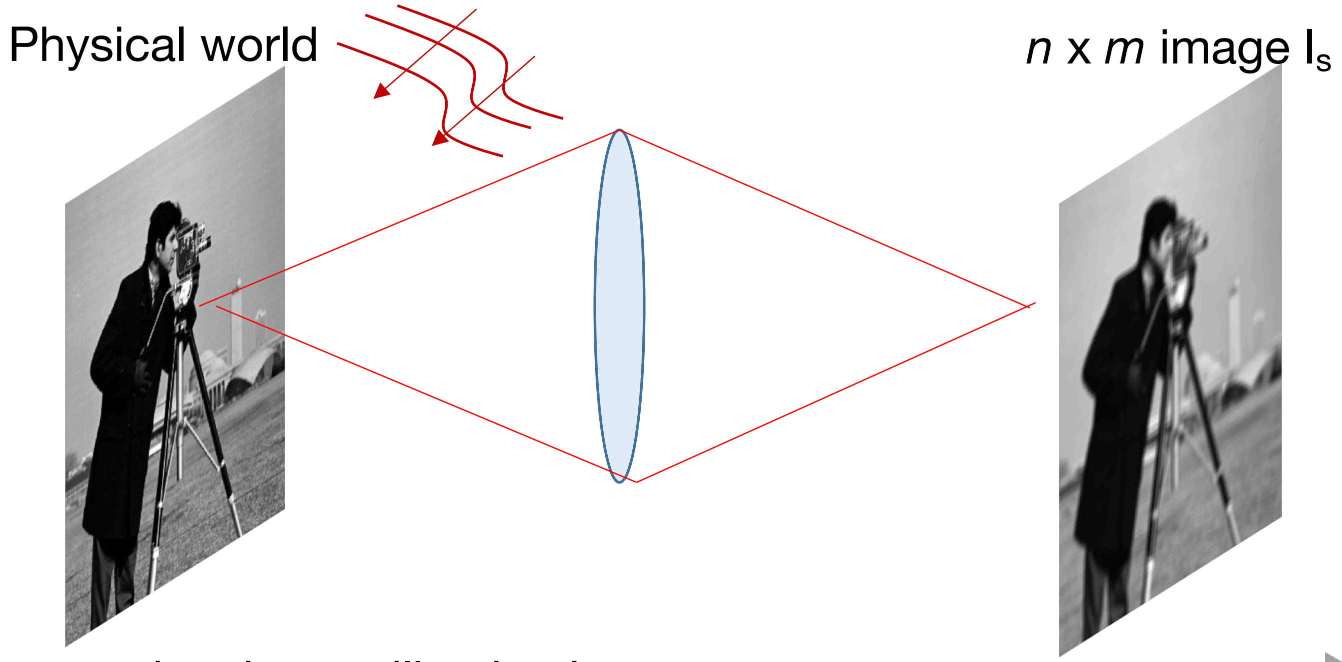
deep imaging



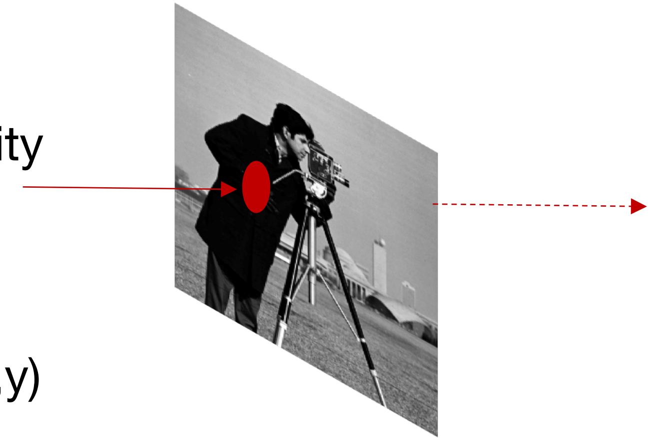
- Assume incoherent illumination
- Assume thin 2D object
- Object is real, non-negative map of absorption/reflectivity



Simple mathematical model of incoherent image formation

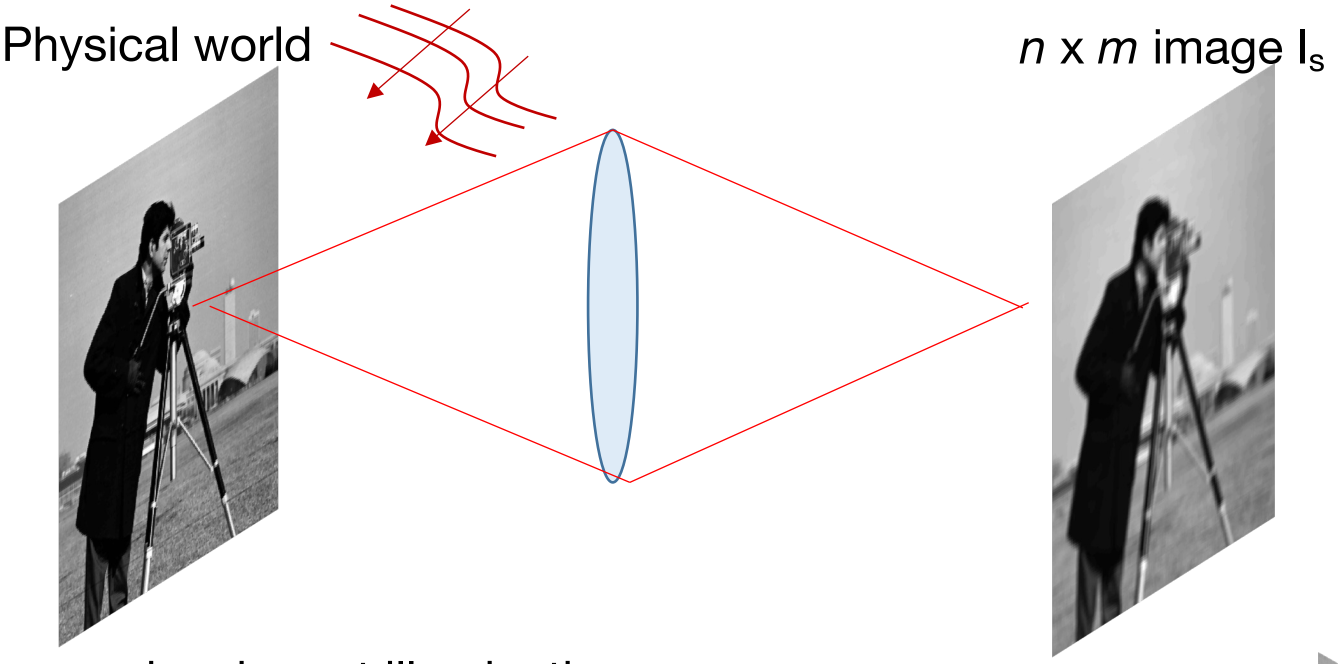


- Assume incoherent illumination
- Assume thin 2D object
- Object is real, non-negative map of absorption/reflectivity

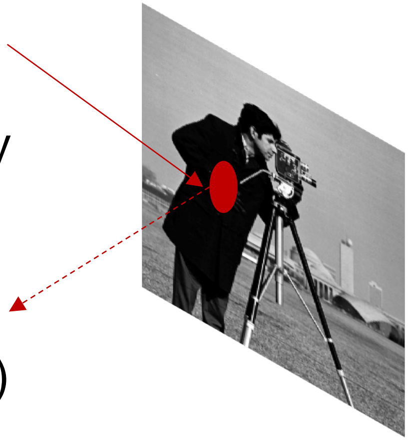


Object transmission: $\mathbf{I_0}(x,y)$
Illumination pattern: $\mathbf{s}(x,y)$
Light exiting object surface: $\mathbf{I_e}(x,y) = \mathbf{I_0}(x,y) \circ \mathbf{s}(x,y)$

Simple mathematical model of incoherent image formation



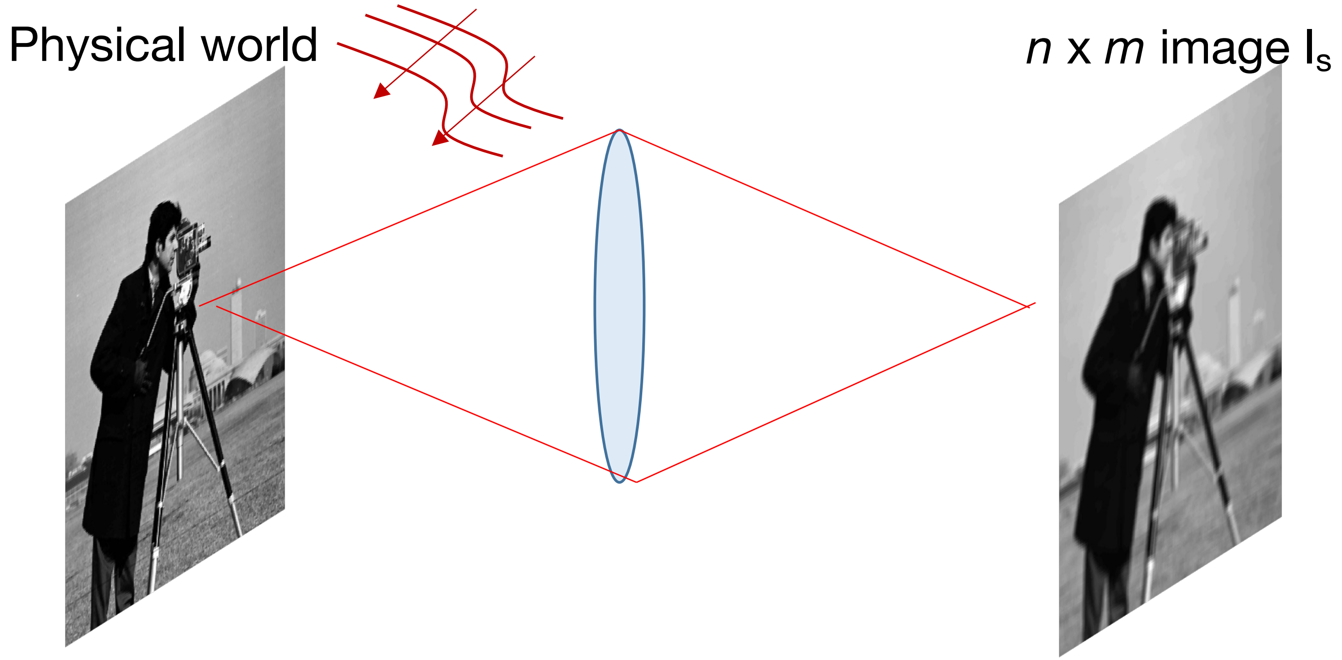
- Assume incoherent illumination
- Assume thin 2D object
- Object is real, non-negative map of absorption/reflectivity



Object *reflectivity*: $\mathbf{I_0}(x,y)$
Illumination pattern: $\mathbf{s}(x,y)$
Light exiting object surface: $\mathbf{I_e}(x,y) = \mathbf{I_0}(x,y) \circ \mathbf{s}(x,y)$

Simple mathematical model of incoherent image formation

deep imaging



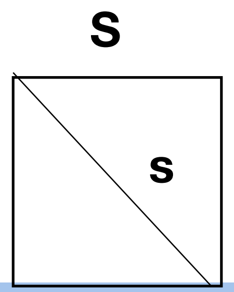
- Assume incoherent illumination
- Assume thin 2D object
- Object is real, non-negative map of absorption/reflectivity

Modeling incoherent illumination

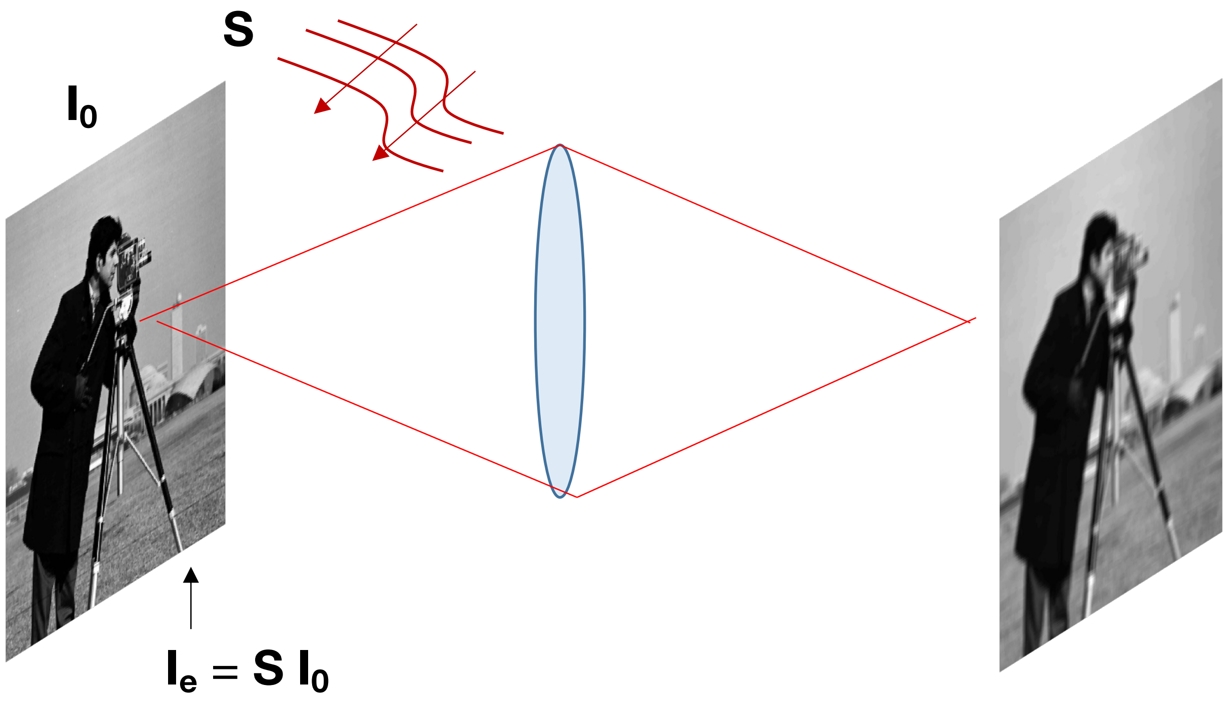
$$\mathbf{I}_e(x,y) = \mathbf{I}_0(x,y) \circ \mathbf{s}(x,y)$$

$$\mathbf{I}_e = \mathbf{S}\,\mathbf{I}_0$$

$\mathrm{diag}(\mathbf{S}) = \mathbf{s}$



Example #1: Optimized illumination pattern (one color)



First, assume perfect camera:
intensity at image plane $\mathbf{I}_p = \mathbf{I}_e = \mathbf{S}\ \mathbf{I}_0$

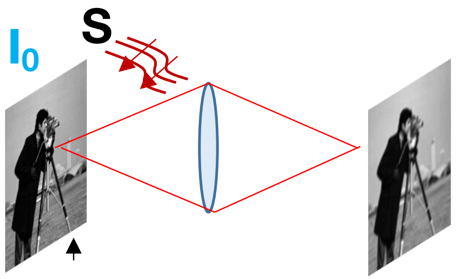
Example #1: Optimized illumination pattern (one color)

deep imaging

Training data:
[$I_0(x, y)$, y]
I_0:100 x 100
Label y: 1x2

Example #1: Optimized illumination pattern (one color)

deep imaging



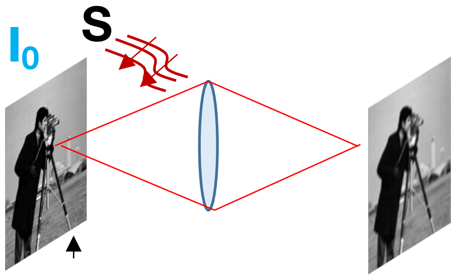
$[I_e(x, y), y]$

Training data:
$[I_0(x, y), y]$
I_0:100 x 100
Label y: 1x2

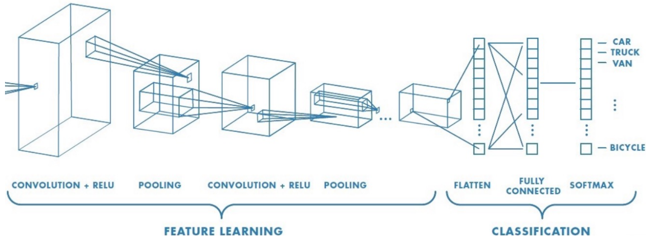
$\boxed{I_e(x,y) = S\, I_0(x,y)}$

Physical Layer

Example #1: Optimized illumination pattern (one color)



$[I_e(x, y), y]$



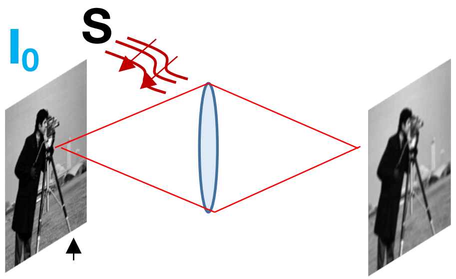
Training data:
$[I_0(x, y), y]$
I_0:100 x 100
Label y: 1x2

$\boxed{I_e(x,y) = \mathbf{S}\, I_0(x,y)}$

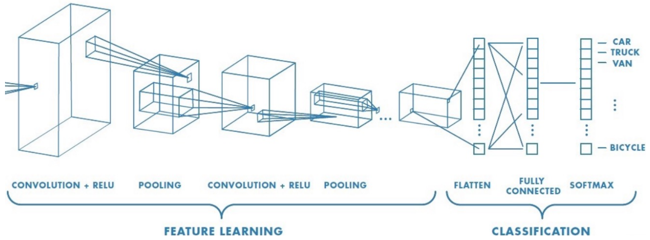
Physical Layer

Task = $\mathbf{W_n} \ldots \mathrm{ReLU}[\mathbf{W_1}\, \mathrm{ReLU}[\mathbf{W_0}\, I_e] \ldots]$

Example #1: Optimized illumination pattern (one color)



$[I_e(x, y), y]$



Training data:
$[I_0(x, y), y]$
I_0:100 x 100
Label y: 1x2

$\boxed{I_e(x,y) = \mathbf{S}\, I_0(x,y)}$

Physical Layer

Task = $\mathbf{W}_n \ldots \mathrm{ReLU}[\mathbf{W}_1 \mathrm{ReLU}[\mathbf{W}_0 I_e] \ldots]$

Tensorflow 1.0 code

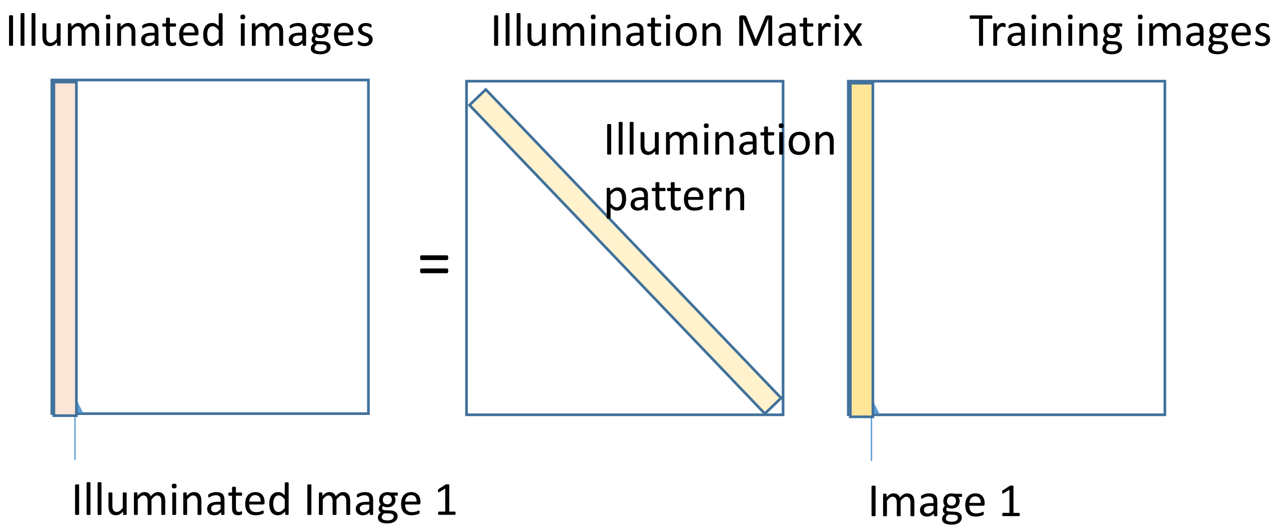
```
training_images = tf.placeholder(tf.float32, [image_size, None])
training_labels = tf.placeholder(tf.float32, [None, 3])
illumination_pattern = tf.truncated_normal([image_size, 1], stddev = 0.1)
illumination_matrix = tf.linalg.diag(illumination_pattern)
illumianted_images = tf.matmul(illumination_matrix, training_images)

#Now, train CNN of your choice using [illumianted_images, training_labels]
#Output of train/test will be CNN weights, classification performance AND illumination_pattern!
```

(Will show CoLab Notebook for implementation in Tensorflow 2.0)

Option 1: tf.linalg.matmul

1.0 code

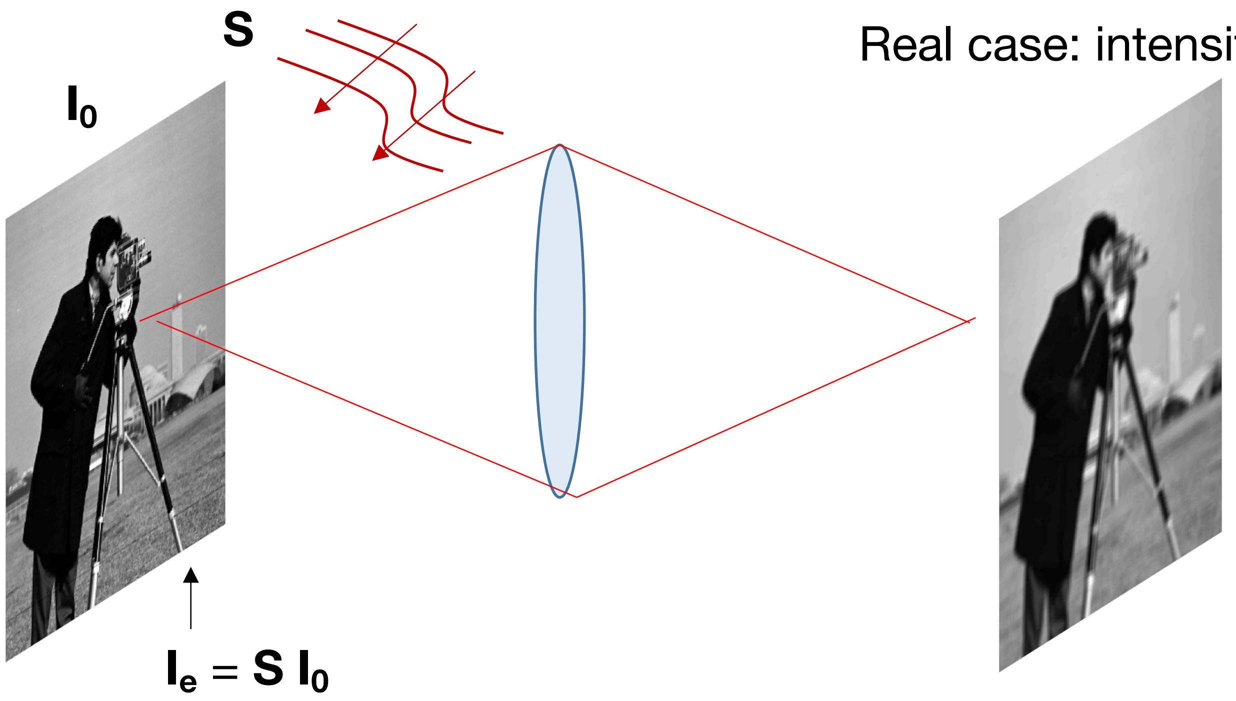
```
training_images = tf.placeholder(tf.float32, [image_size, None])
training_labels = tf.placeholder(tf.float32, [None, 3])
illumination_pattern = tf.truncated_normal([image_size, 1], stddev = 0.1)
illumination_matrix = tf.linalg.diag(illumination_pattern)
illumianted_images = tf.matmul(illumination_matrix, training_images)

#Now, train CNN of your choice using [illumianted_images, training_labels]
#Output of train/test will be CNN weights, classification performance AND illumination_pattern!
```



Option 2: tf.linalg.multiply (will show in CoLab Notebook)

Simple mathematical model of image formation

Real case: intensity at image plane $\mathbf{I_p}$ = **blurred**



Simple mathematical model of image formation

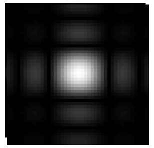
deep imaging

Lenses blur and rescale images:
(We'll learn how exactly next few weeks)

Input intensity

$*$

Convolution filter h



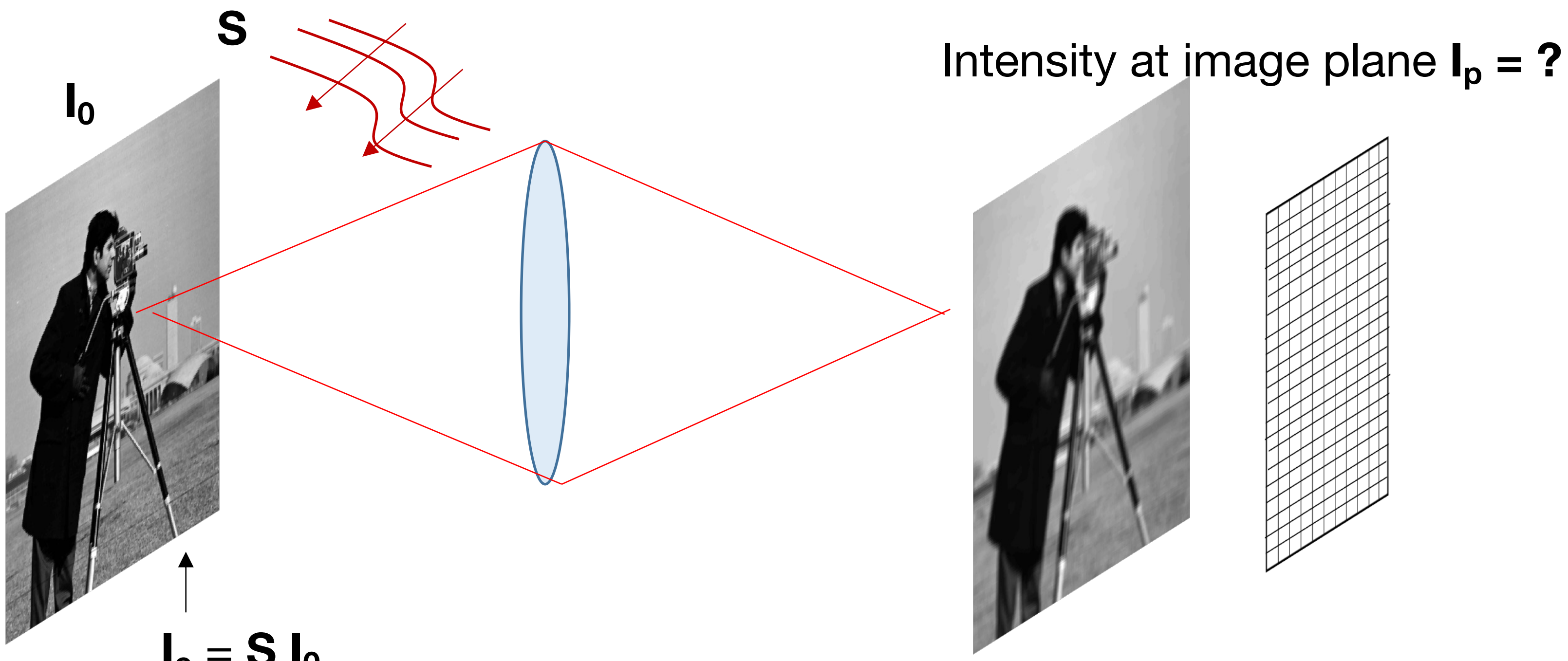
$=$

Output intensity

$\mathbf{I_e}(x/M,y/M) \quad * \quad \mathbf{h}(x,y) \quad = \quad \mathbf{I_p}(x,y)$

Assuming we've resized by M, $\mathbf{I_p} = \mathbf{I_e} * \mathbf{h} = \mathbf{H}\,\mathbf{I_e}$

Simple mathematical model of image formation

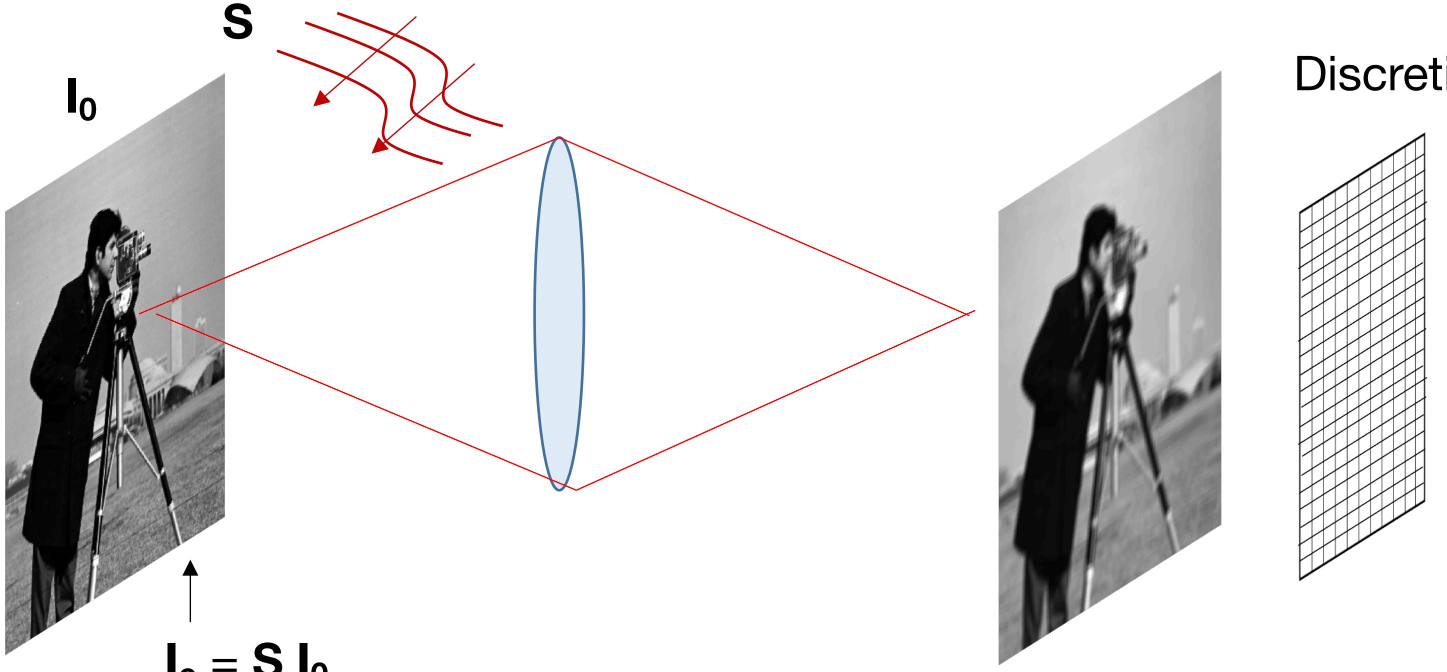


$\mathbf{I}_e = \mathbf{S}\,\mathbf{I}_0$

$\mathbf{I}_p = \mathbf{H}\,\mathbf{I}_e = \mathbf{H}\,\mathbf{S}\,\mathbf{I}_0$

Simple mathematical model of image formation

Discretization by detector **?**



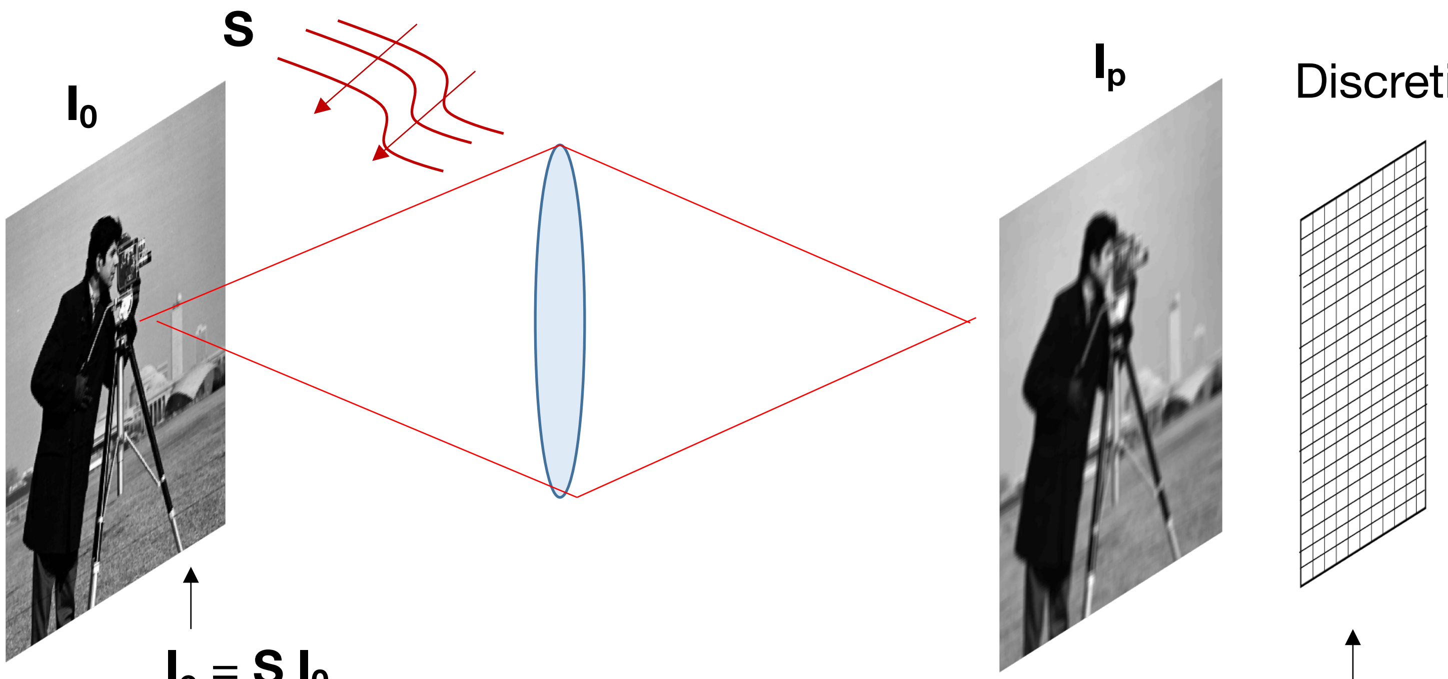
$\mathbf{I_e} = \mathbf{S}\,\mathbf{I_0}$

$\mathbf{I_p} = \mathbf{H}\,\mathbf{I_e} = \mathbf{H}\,\mathbf{S}\,\mathbf{I_0}$

Use downsampling matrix (sum-pooling)

$$\mathbf{D} = \begin{bmatrix} 0.5 & 0.5 & 0 & 0 & 0 & \dots & & & & \\ & & 0.5 & 0.5 & 0 & 0 & 0 & \dots & & \\ & & & & 0.5 & 0.5 & 0 & 0 & 0 & \dots \\ & & \vdots & & & & \vdots & & & \end{bmatrix}$$

Simple mathematical model of image formation

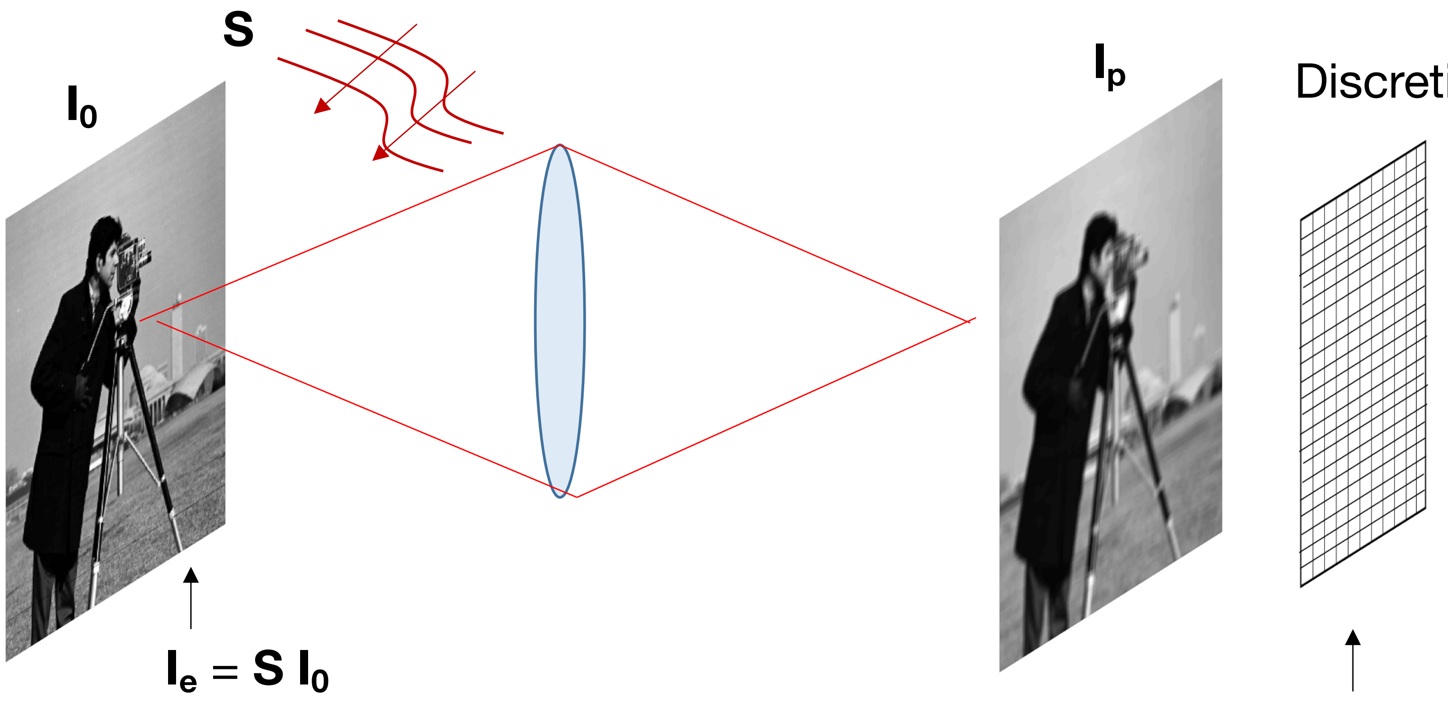


$$\boxed{I_s = D\, I_p = D\, H\, S\, I_0}$$

Use downsampling matrix (sum-pooling)

$$D = \begin{bmatrix} 0.5 & 0.5 & 0 & 0 & 0 & \cdots & & & \\ & & 0.5 & 0.5 & 0 & 0 & 0 & \cdots & \\ & & & & 0.5 & 0.5 & 0 & 0 & 0 \; \cdots \\ & \vdots & & & & \vdots & & & \end{bmatrix}$$

Simple mathematical model of image formation

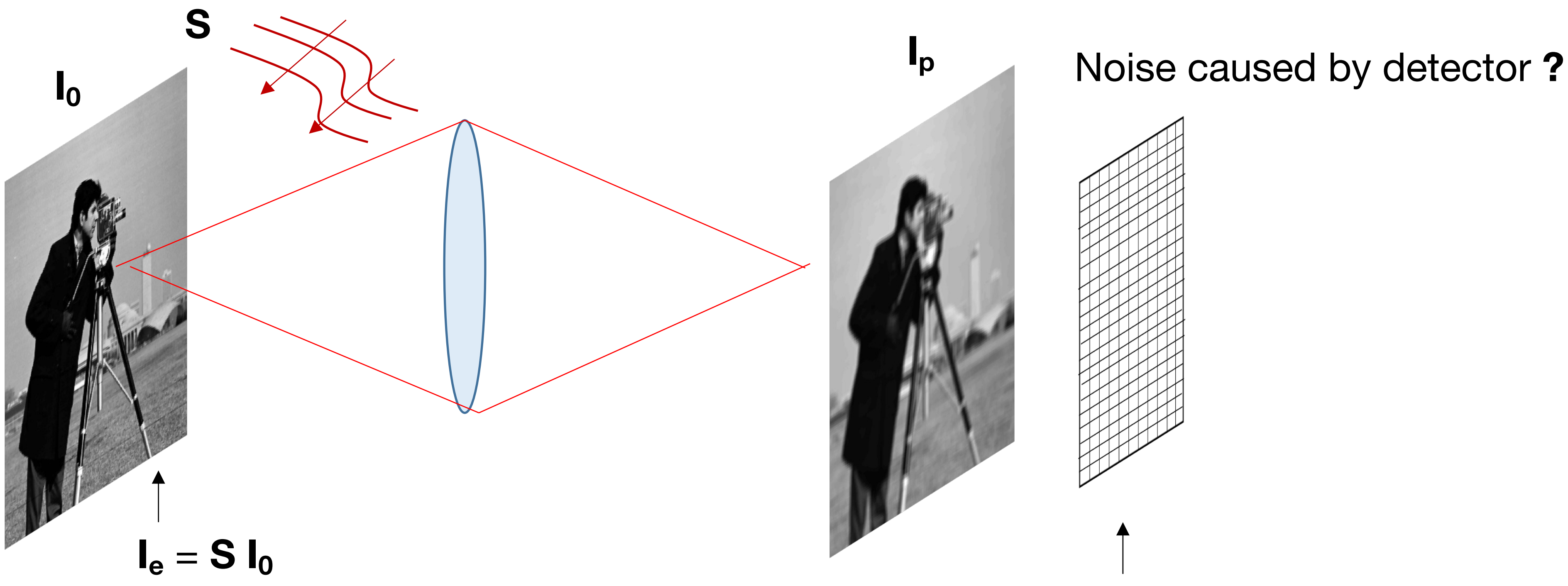


$$\boxed{I_s = D\, I_p = D\, H\, S\, I_0}$$

$$D = \begin{bmatrix} w1 & w2 & 0 & 0 & 0 & \dots & & & & & & \\ & & w3 & w4 & 0 & 0 & 0 & \dots & & & & \\ & & & & w5 & e6 & 0 & 0 & 0 & \dots & & \\ & \vdots & & & & & \vdots & & & & & \end{bmatrix}$$

*Can make these learnable weights

Simple mathematical model of image formation



$$\boxed{I_s = D\, I_p + N}$$

Can also add in detector-dependent noise N = k * np.random.randn(dx, dy)

(zero-mean Gaussian noise, for example)

Pause to take a look at:

physical_layers_example.ipynb

Physical Layers

Digital Layers

deep imaging

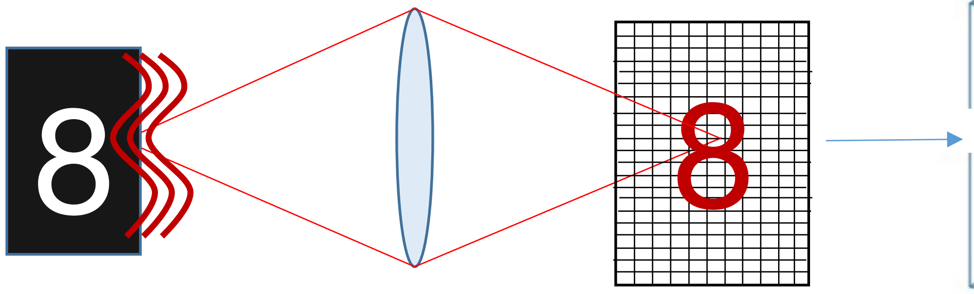
Physical world

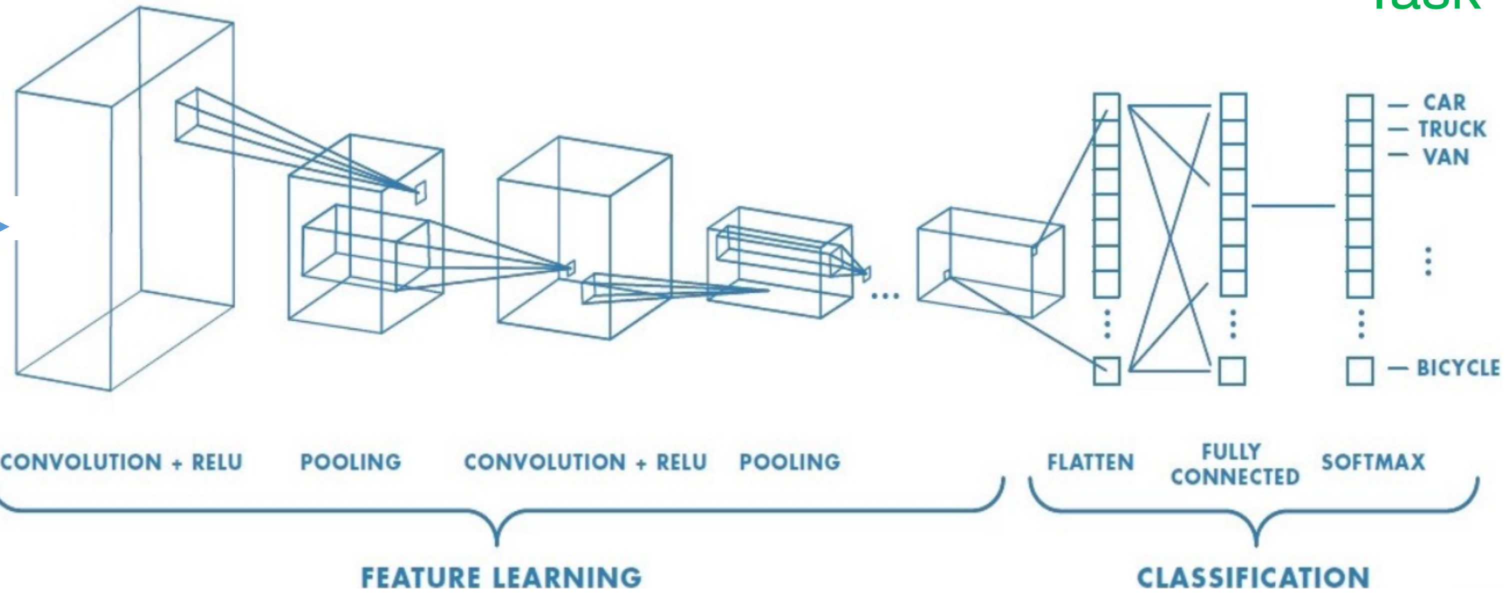
$\mathbf{I}_0(x_0,y_0)$

Digital Image

$\mathbf{I}_s(x,y)$

Task





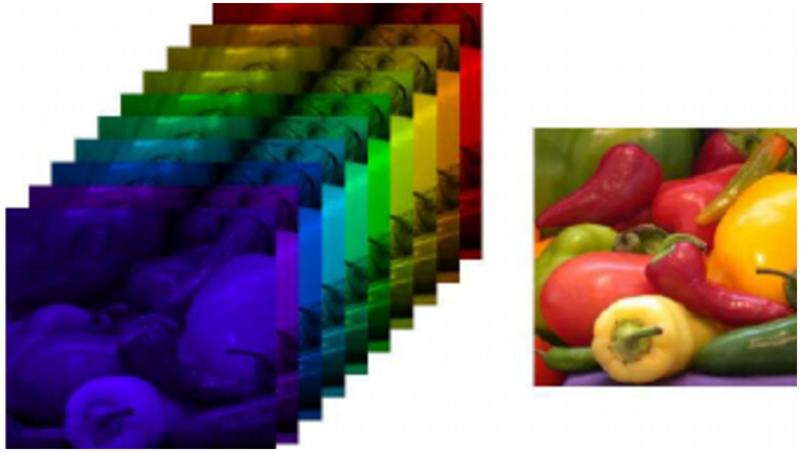
Digital layers

Physical layers

$$\text{Task} = \mathbf{W}_n \ldots \mathrm{ReLU}[\mathbf{W}_1\, \mathrm{ReLU}[\mathbf{W}_0\, f[\mathbf{I}_0]]\ldots]$$

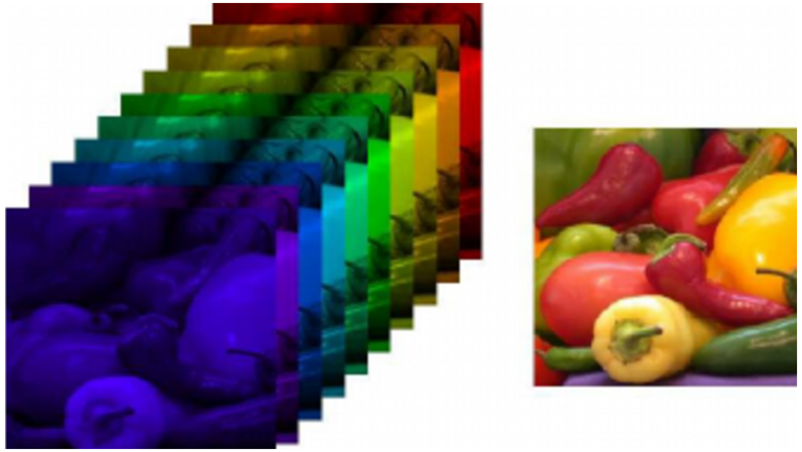
$$\text{Task} = \mathbf{W}_n \ldots \mathrm{ReLU}[\mathbf{W}_1\, \mathrm{ReLU}[\mathbf{W}_0\, \mathbf{D}\,\mathbf{H}\,\mathbf{S}\,\mathbf{I}_0]\ldots]$$

Example #2: Optimized color filter for a grayscale camera

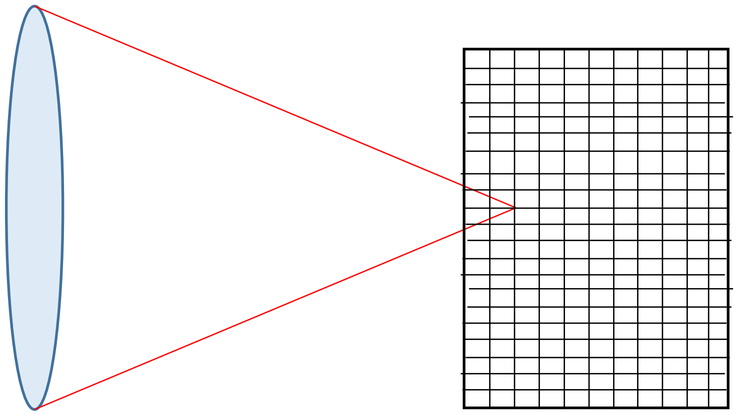


“Ground truth” object:
$I_0(x, y, \lambda)$
100 x 100 pix. x 30 spectral channels

Example #2: Optimized color filter for a grayscale camera



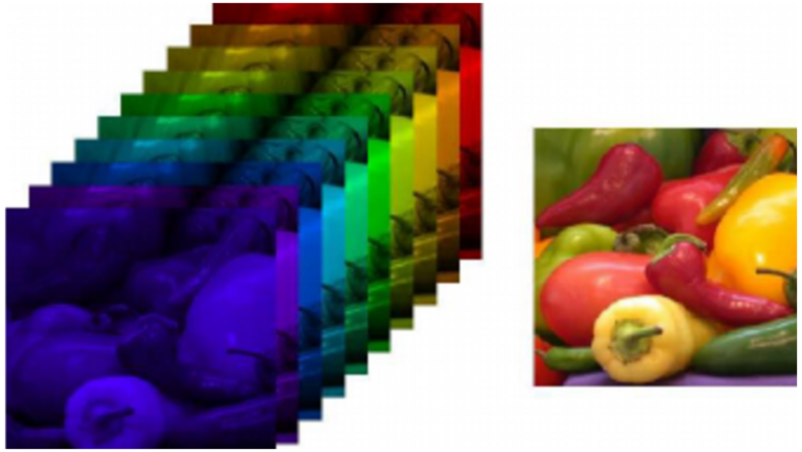
“Ground truth” object:
$I_0(x, y, \lambda)$
100 x 100 pix. x 30 spectral channels

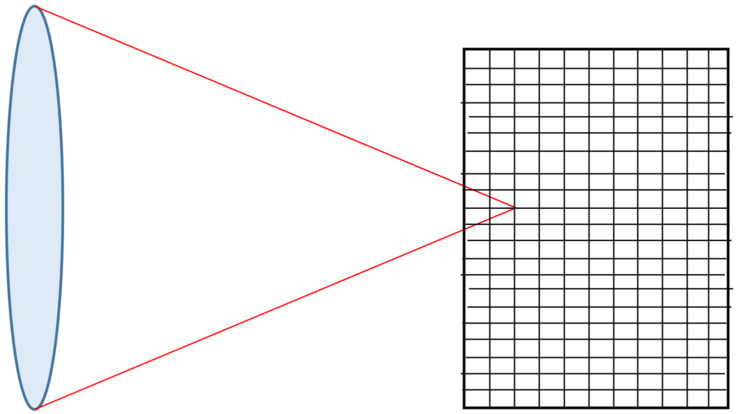


Monochromatic
camera sensor

deep imaging

Example #2: Optimized color filter for a grayscale camera

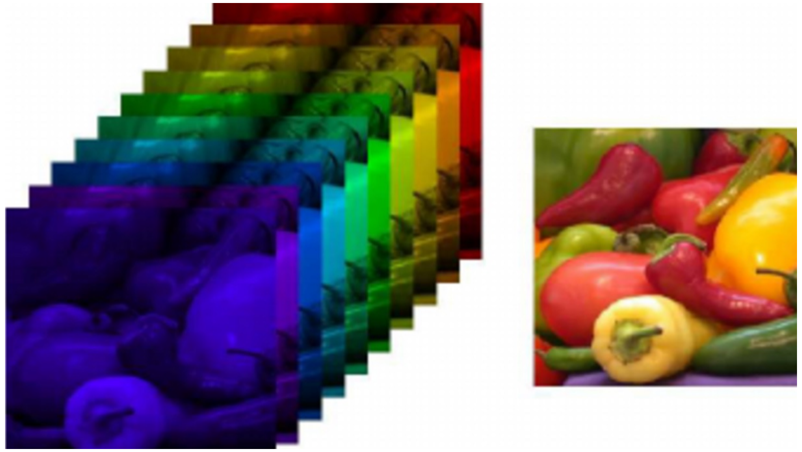


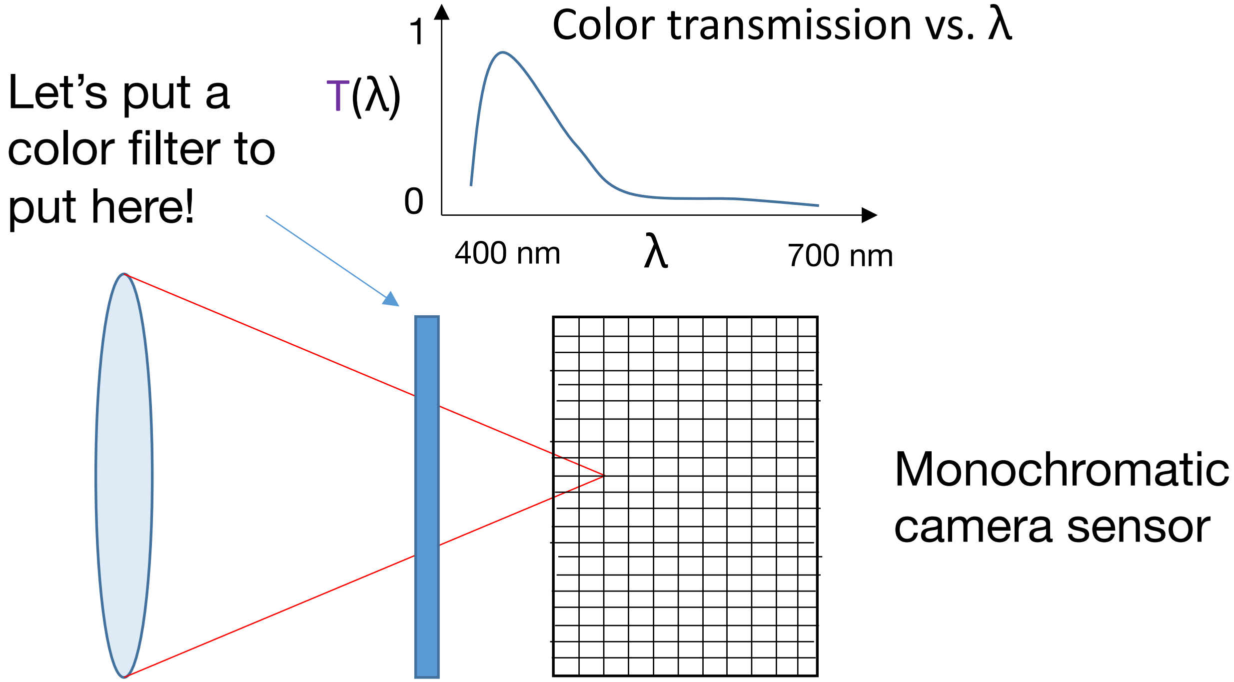


"Ground truth" object:
$I_0(x, y, \lambda)$
100 x 100 pix. x 30 spectral channels

$$\boxed{I_s(x, y) = \sum_{\lambda} I_0(x, y, \lambda)}$$

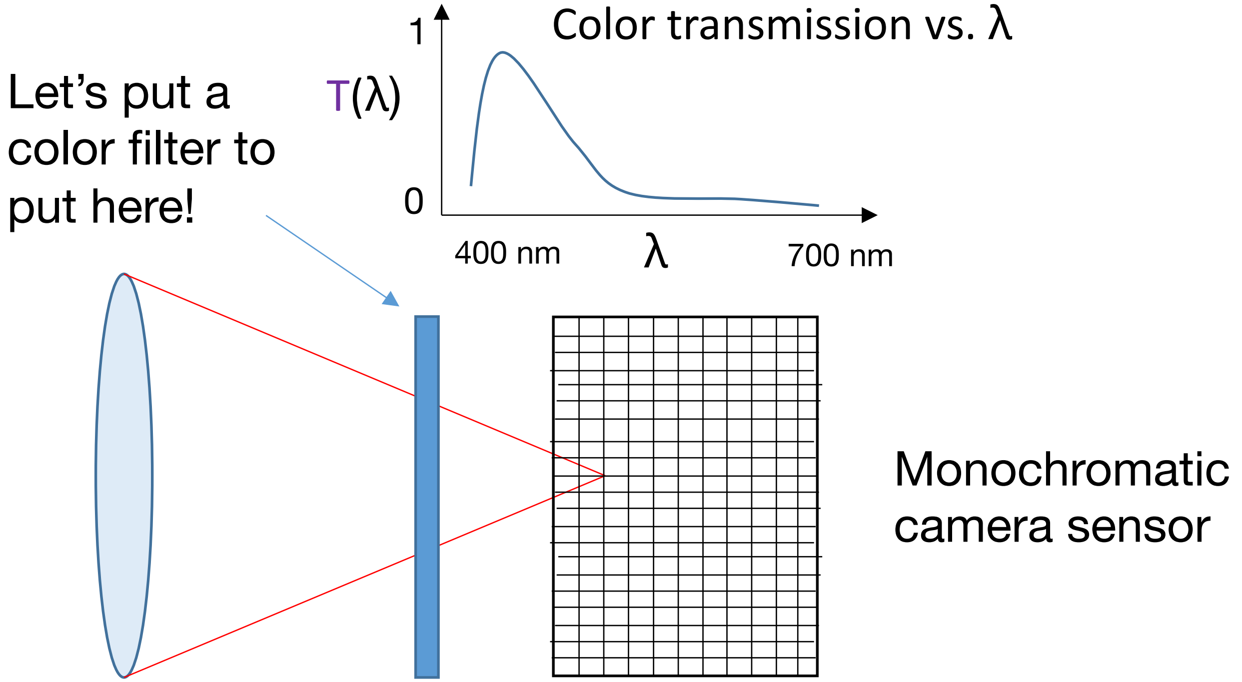
Example #2: Optimized color filter for a grayscale camera





"Ground truth" object:
$I_0(x, y, \lambda)$
100 x 100 pix. x 30 spectral channels

Example #2: Optimized color filter for a grayscale camera

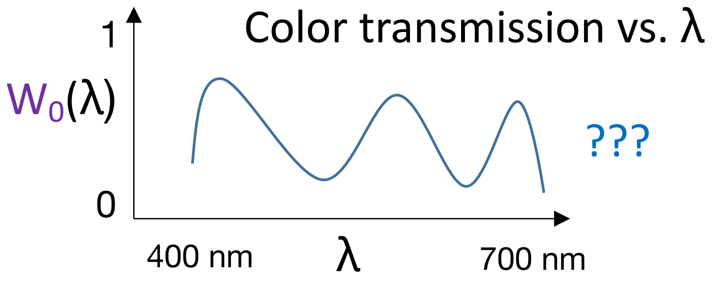


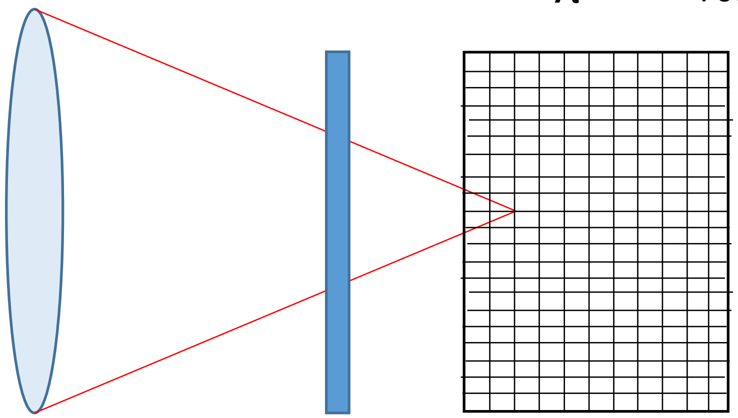
"Ground truth" object:
$I_0(x, y, \lambda)$
100 x 100 pix. x 30 spectral channels

$$I_s(x, y) = \sum_{\lambda} T(\lambda)\, I_0(x, y, \lambda)$$

Example #2: Optimized color filter for a grayscale camera

Design optimal color filter for classification:





Monochromatic camera sensor

Training data:
[$I_0(x, y, \lambda)$, y]
I_0:100 x 100 pix. x 30
Label y: 1x3 - pepper, broccoli, green beans

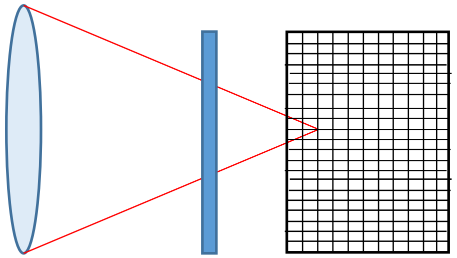
$$I_s(x, y) = \sum_{\lambda} W_0(\lambda)\, I_0(x, y, \lambda)$$

Physical Layer

Example #2: Optimized color filter for a grayscale camera

Training data:
[$I_0(x, y, \lambda)$, y]
I_0:100 x 100 x 30
Label y: 1x3

Example #2: Optimized color filter for a grayscale camera



Training data:

$[I_0(x, y, \lambda), y]$

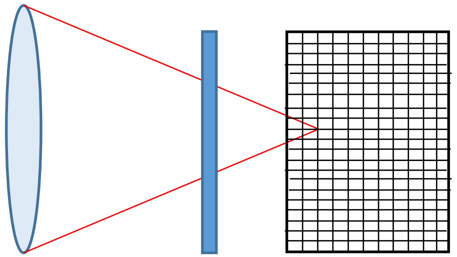
I_0: 100 x 100 x 30

Label y: 1x3

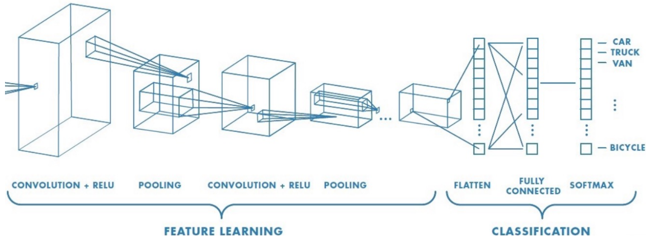
$$I_s(x, y) = \sum_{\lambda} W_0(\lambda)\, I_0(x, y, \lambda)$$

Physical Layer

Example #2: Optimized color filter for a grayscale camera



$[I_s(x, y), y]$



Training data:

$[I_0(x, y, \lambda), y]$

I_0: 100 x 100 x 30

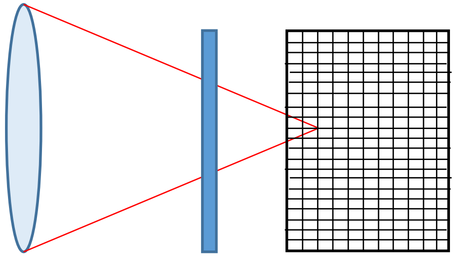
Label y: 1x3

$$\boxed{I_s(x, y) = \sum_{\lambda} W_0(\lambda)\, I_0(x, y, \lambda)}$$

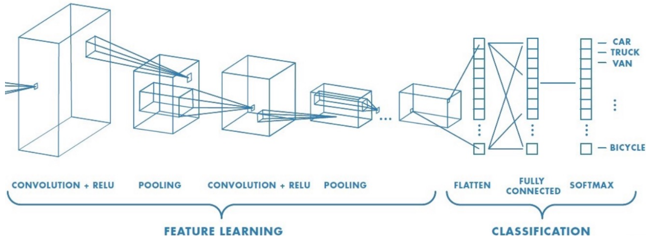
Physical Layer

$$\text{Task} = \mathbf{W_n} \ldots \mathrm{ReLU}[\mathbf{W_1}\, \mathrm{ReLU}[\mathbf{W_0}\, I_s] \ldots]$$

Example #2: Optimized color filter for a grayscale camera



[$I_s(x, y)$, y]



Training data:

[$I_0(x, y, \lambda)$, y]

I_0:100 x 100 x 30

Label y: 1x3

$$\boxed{I_s(x, y) = \sum_{\lambda} W_0(\lambda)\, I_0(x, y, \lambda)}$$

Physical Layer

Task = $\mathbf{W_n} \ldots \text{ReLU}[\mathbf{W_1}\, \text{ReLU}[\mathbf{W_0}\, I_s] \ldots]$

```
multispectral_data = tf.placeholder(tf.float32, [None, num_colors, image_size])
veg_labels = tf.placeholder(tf.float32, [None, 3])
filter_weights = tf.truncated_normal([num_colors, 1], stddev = 0.1)
filtered_images = tf.einsum('aij,jk->aik', multispectral_data, filter_weights)

#Now, train CNN of your choice using [filtered_images, veg_labels]
#Output of training/testing will be CNN weights, classification performance AND filter_weights!
```

Example implementation with Tensorflow 1.0 code

Tensorflow: operations to sum along 3rd (or higher) dimension

Option 1: Einsum (shown as Tensorflow 1.0 code, and also applicable in Tensorflow 2.0)

```
filtered_images = tf.einsum('aij,jk->aik', multispectral_data, filter_weights)
```

Option 2: tf.reduce_sum

```
filtered_images = tf.reduce_sum(multispectral_data * filter_weights, axis=2)
```

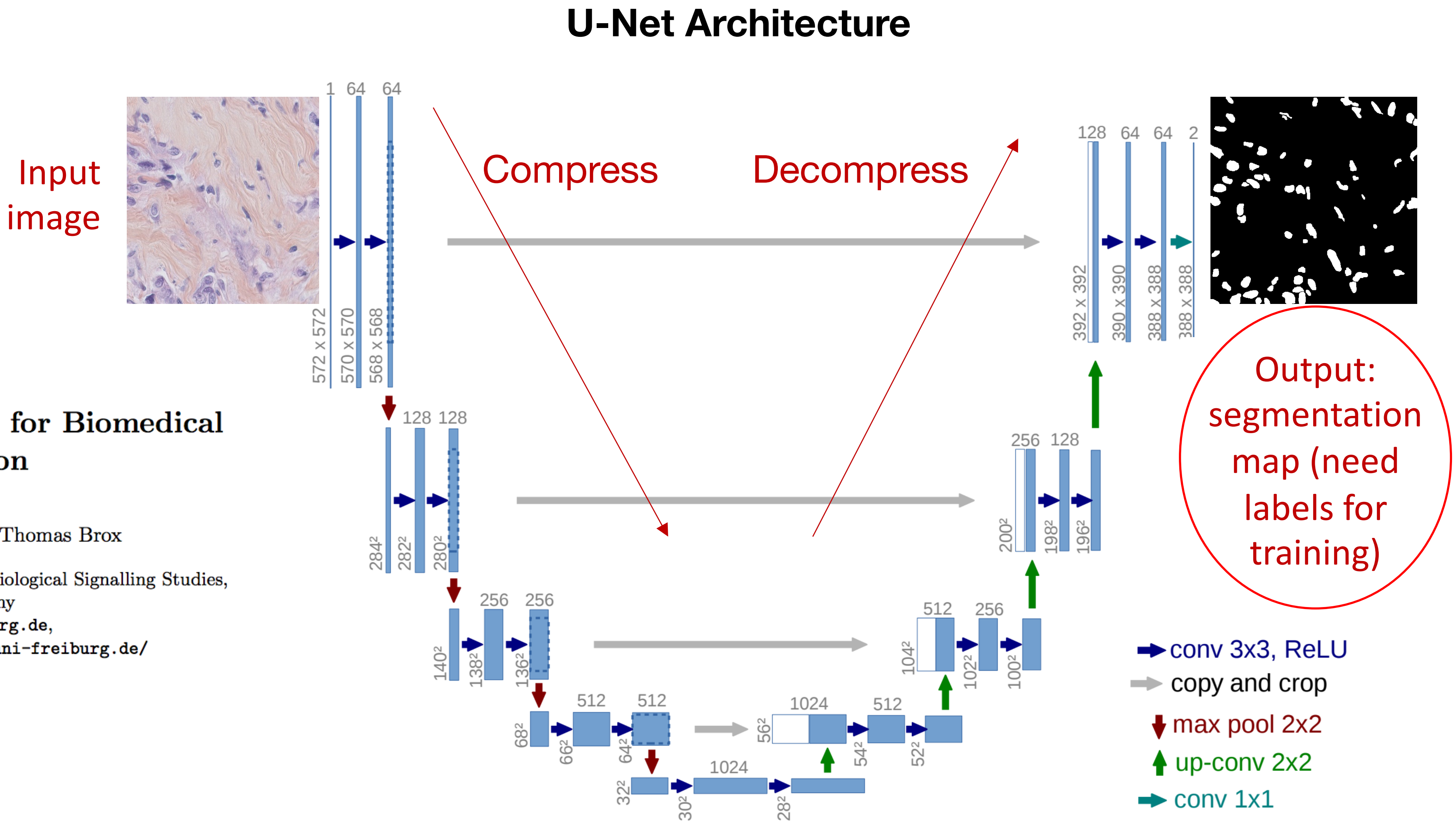
Option 3: Locally connected conv2D with a 1x1 filter size

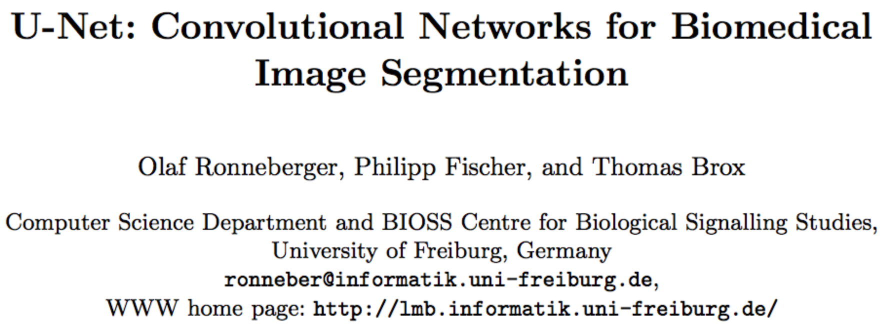
https://github.com/keras-team/keras/blob/master/keras/layers/local.py#L183

Pause to take a look at:

weighted_image_sum_example.ipynb

Example 3: learned illumination pattern for improved segmentation



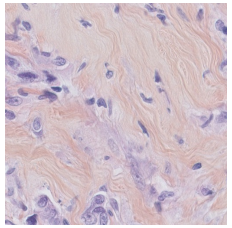
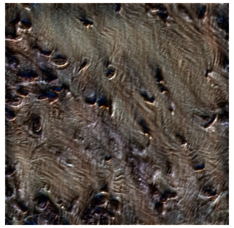
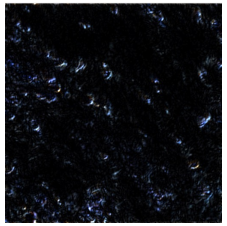

U-Net: Convolutional Networks for Biomedical Image Segmentation

Olaf Ronneberger, Philipp Fischer, and Thomas Brox

Computer Science Department and BIOSS Centre for Biological Signalling Studies, University of Freiburg, Germany
ronneber@informatik.uni-freiburg.de,
WWW home page: http://lmb.informatik.uni-freiburg.de/

Example 3: learned illumination pattern for improved segmentation

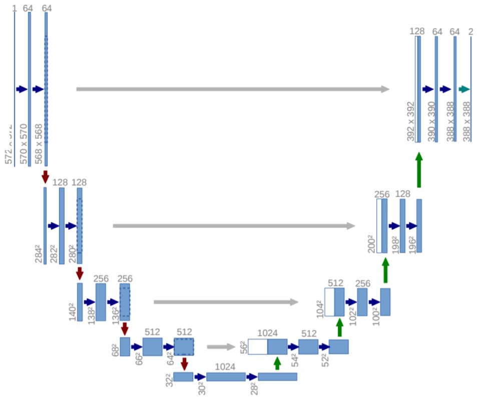
Variably illuminated images from different LEDs

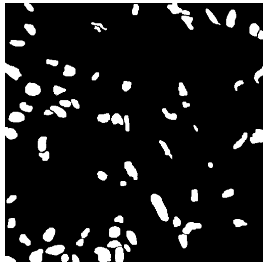
…

$w_1 I_1 \quad + \quad w_2 I_2 \quad + \quad w_3 I_3 \quad + \dots$

U-Net CNN



Segmentation Map



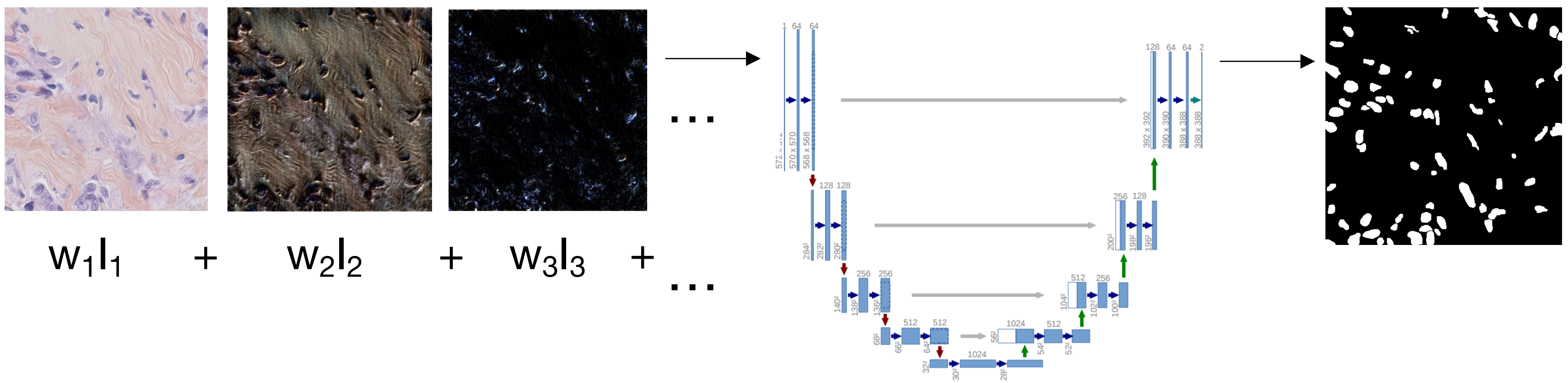
*If we allow w's here to be trainable weights, then we can find ideal brightnesses for different LEDs to illuminate a sample of interest!

Example 3: learned illumination pattern for improved segmentation

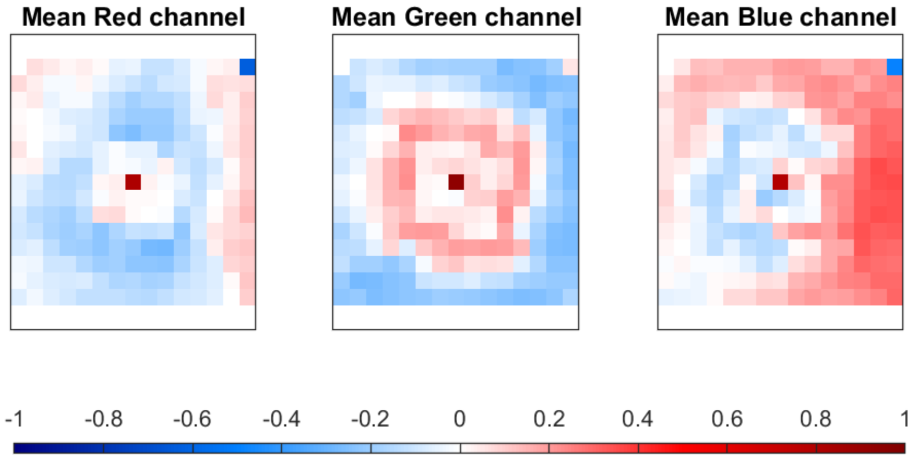
Variably illuminated images

U-Net CNN

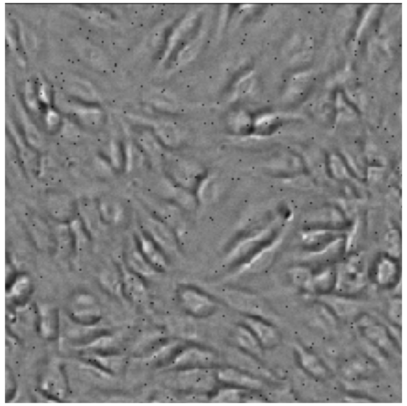
Segmentation Map



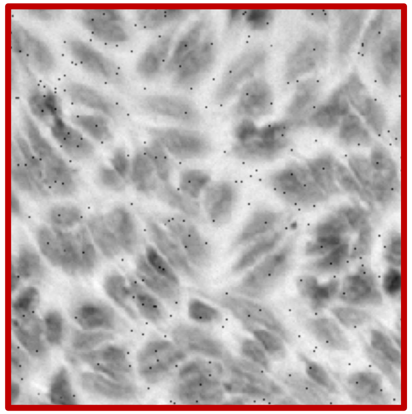
Optimized illumination for nuclei segmentation



Standard illumination



Learned illumination

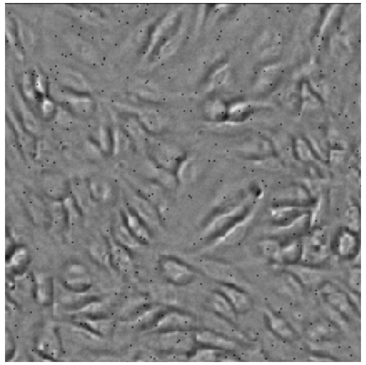


+5-10% accuracy

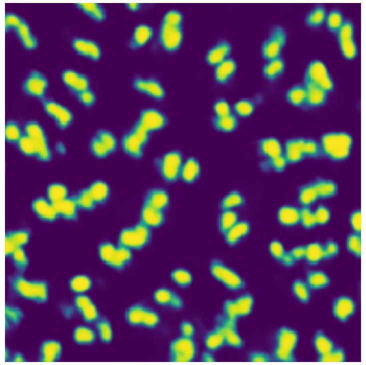
See C. Cooke et al., "Physics-enhanced machine learning for virtual fluorescence microscopy," ICCV (2021)

Image segmentation –current workflow

Capture: BF images



Capture: Fluorescence



(e.g., DAPI-stained nuclei)

Threshold

Segmentation Mask

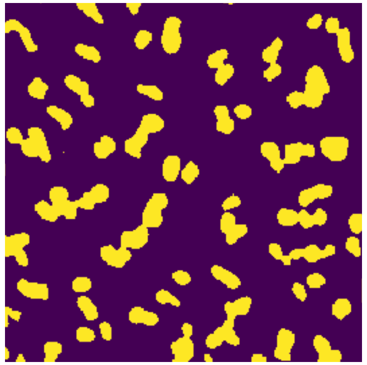
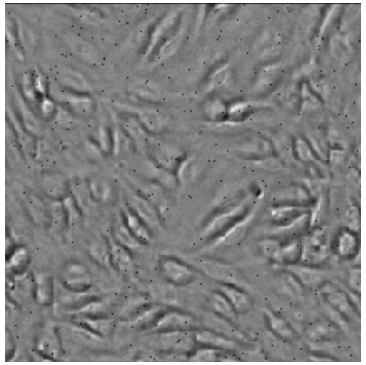
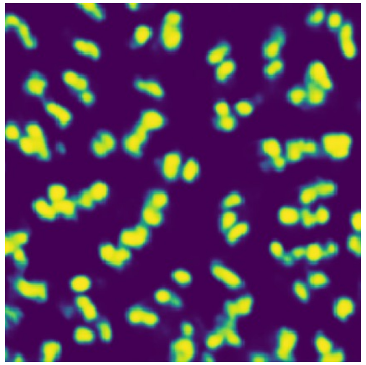


Image segmentation –current workflow

Capture: BF images



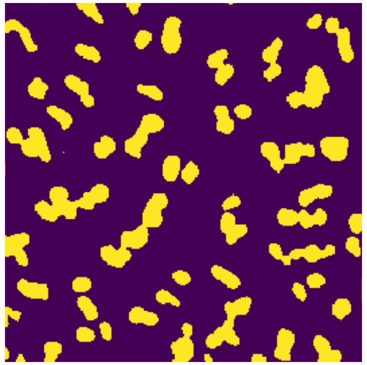
Capture: Fluorescence



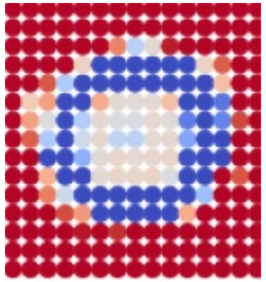
(e.g., DAPI-stained nuclei)

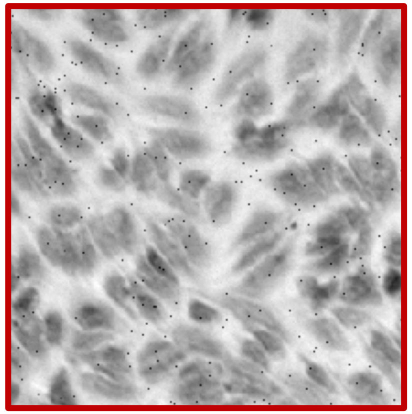
Threshold

Segmentation Mask



Learned illumination





Optimally illuminated

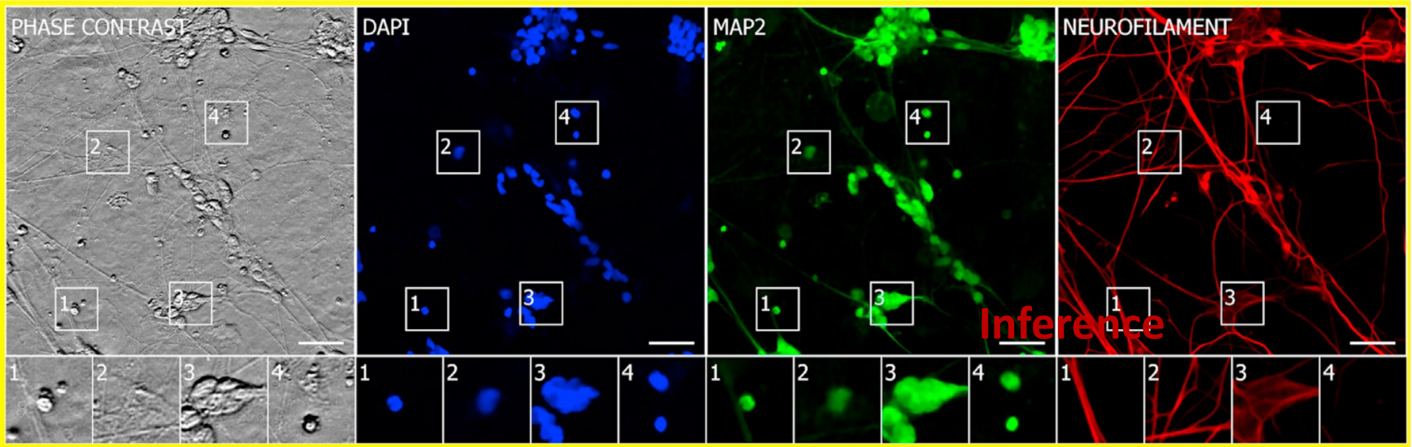
Inference via a trained U-Net

deep imaging

in silico labeling: fluorescence image inference from bright-field data

In Silico Labeling: Predicting Fluorescent Labels in Unlabeled Images

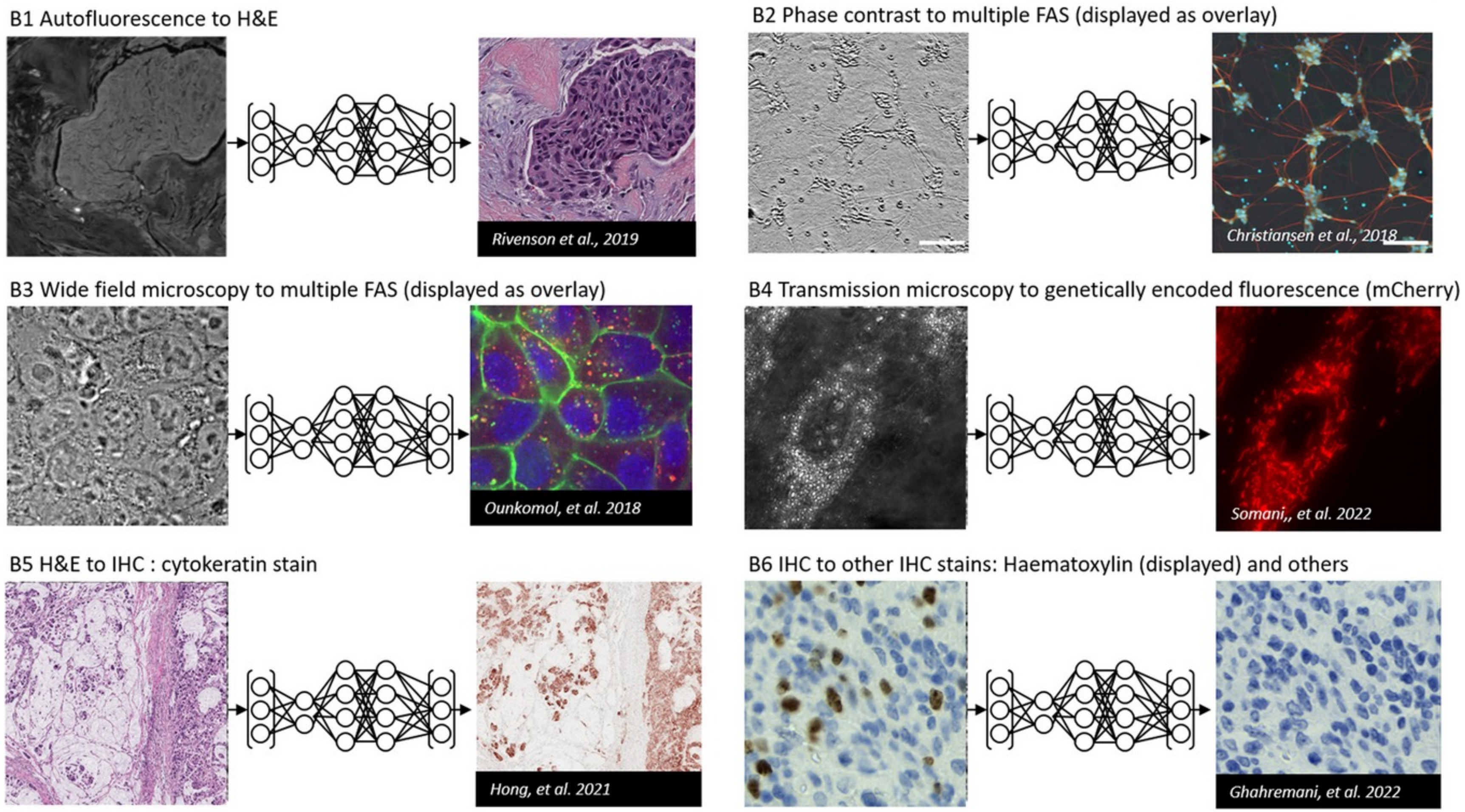
Eric M. Christiansen,[1,11,*] Samuel J. Yang,[1] D. Michael Ando,[1,9] Ashkan Javaherian,[2,9] Gaia Skibinski,[2,9] Scott Lipnick,[3,4,8,9] Elliot Mount,[2,10] Alison O'Neil,[3,10] Kevan Shah,[2,10] Alicia K. Lee,[2,10] Piyush Goyal,[2,10] William Fedus,[1,6,10] Ryan Poplin,[1,10] Andre Esteva,[1,7] Marc Berndl,[1] Lee L. Rubin,[3] Philip Nelson,[1,*] and Steven Finkbeiner[2,5,*]
[1]Google, Inc., Mountain View, CA 94043, USA
[2]Taube/Koret Center for Neurodegenerative Disease Research and DaedalusBio, Gladstone Institutes, San Francisco, CA 94158, USA
[3]Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA 02138, USA
[4]Department of Biomedical Informatics, Harvard Medical School, Boston, MA 02115, USA
[5]Departments of Neurology and Physiology, University of California, San Francisco, 94158, USA
[6]Montreal Institute of Learning Algorithms, University of Montreal, Montreal, QC, Canada
[7]Department of Electrical Engineering, Stanford University, Stanford, CA 94305, USA
[8]Center for Assessment Technology and Continuous Health, Massachusetts General Hospital, Boston, MA 02114, USA



BF Focal stack
(26+ images)

Task: bright-field to fluorescence image Inference

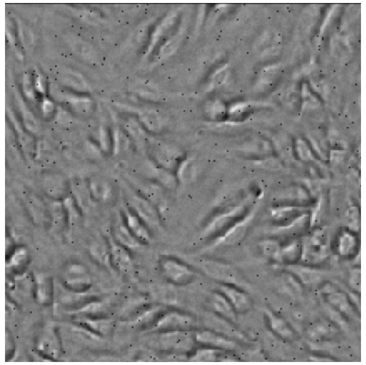
“Virtual Staining” – can be used to covert one image type to many others



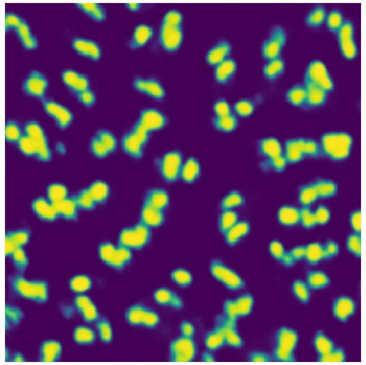
L. Kreiss et al., “Digital staining in optical microscopy using deep learning--a review,” Photonix (2024)
B. Bai et al., “Deep learning-enabled virtual histological staining of biological samples,” Nature Light Sci (2023)

Image segmentation versus *in silico* labeling (fluorescence inference)

Capture: Bright field



Capture: Fluorescence



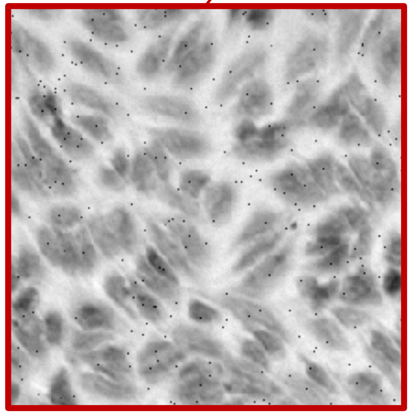
Threshold

Segmentation Mask

Directly infer the fluorescence image

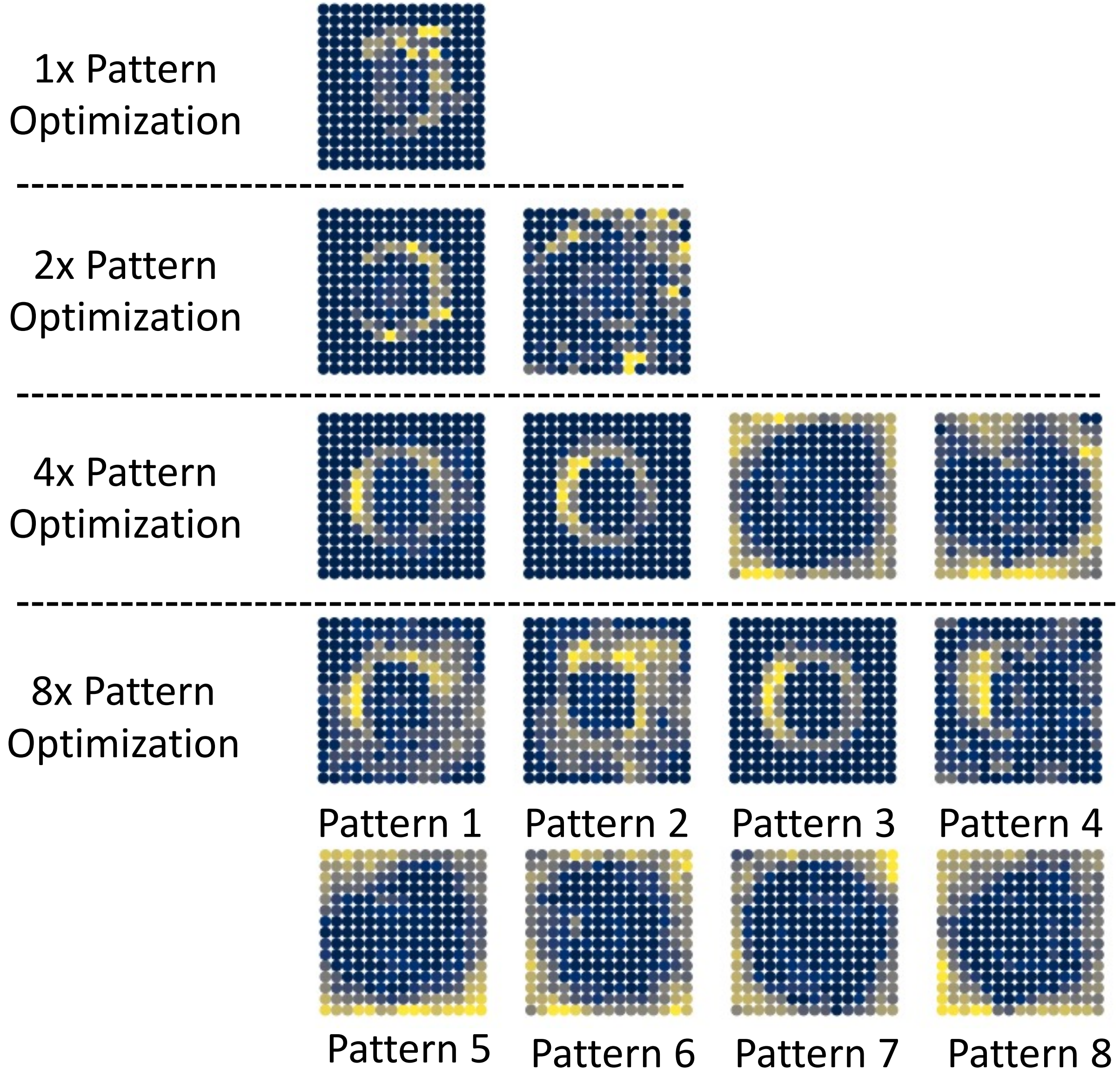
Learned illumination for fluorescent image inference?

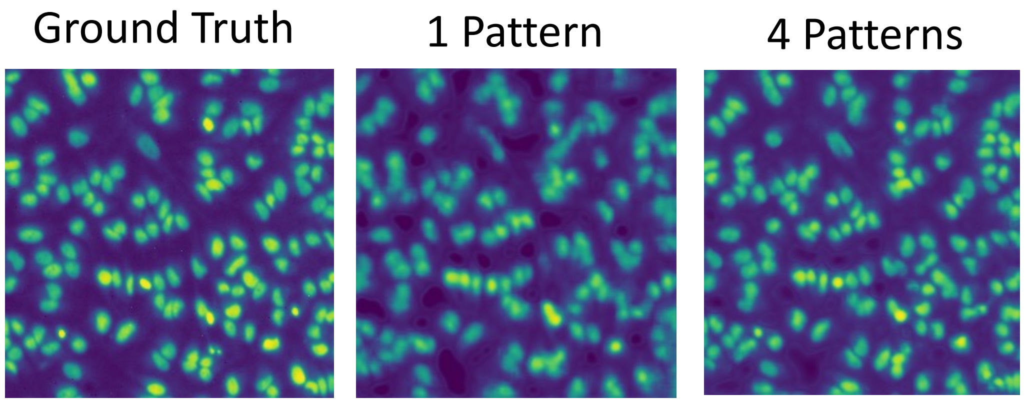
?

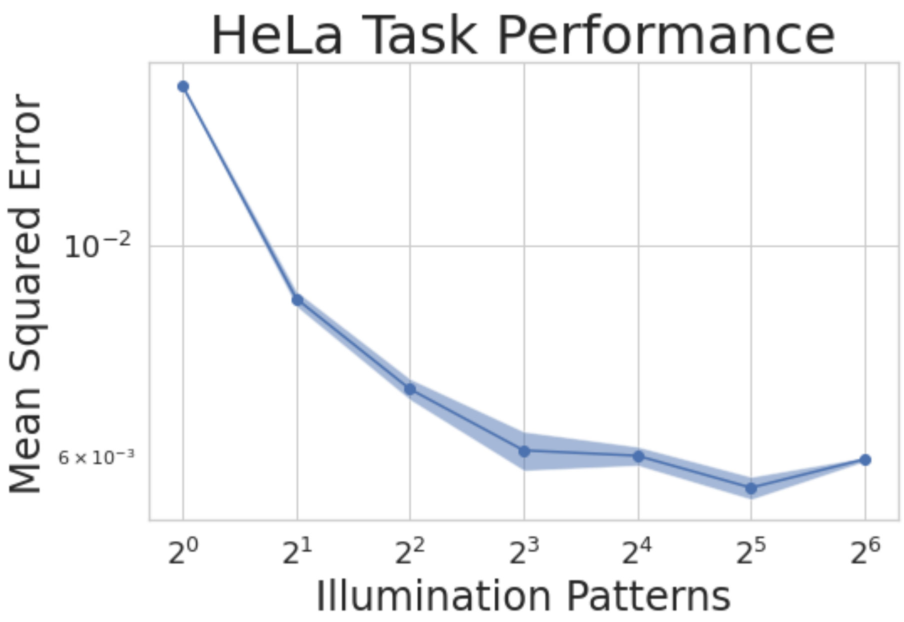


Optimally illuminated

Multiple Patterns for Fluorescence image inference







See C. Cooke et al., "Physics-enhanced machine learning for virtual fluorescence microscopy," ICCV (2021)

Summary: simple physical layers for incoherent imaging

deep imaging

- Deal with sample/image intensities I, real and non-negative

- Effect of illumination is element-wise multiplication $I_e(x,y) = \mathbf{S}\ I_0(x,y)$

λ

- Imaging systems blur the object via point-spread function matrix **H**

$I_b(x,y) = \mathbf{H}\ I_0(x,y)$

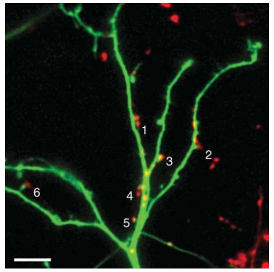
- Discrete pixels down-sample the object via $I_d(x,y) = \mathbf{D}\ I_0(x,y)$

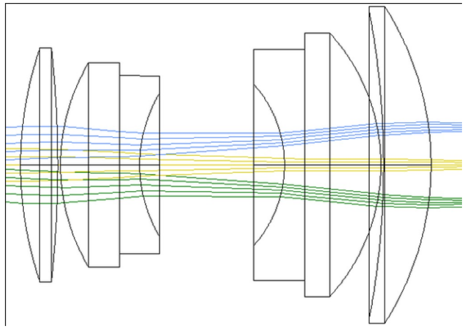
- Add noise into measurement $I_N(x,y) = \mathbf{D}\ I_0(x,y) + \mathbf{N}$

- Different colors add linearly $I_s(x, y) = \Sigma\ I_0(x, y, \lambda)$

What is light and how can we model it?

- Interpretation #1: Radiation (*Incoherent*)
- Model: Rays

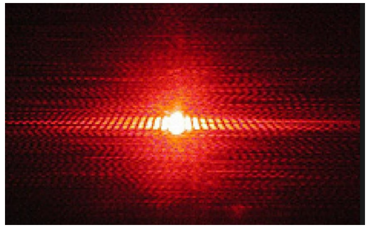


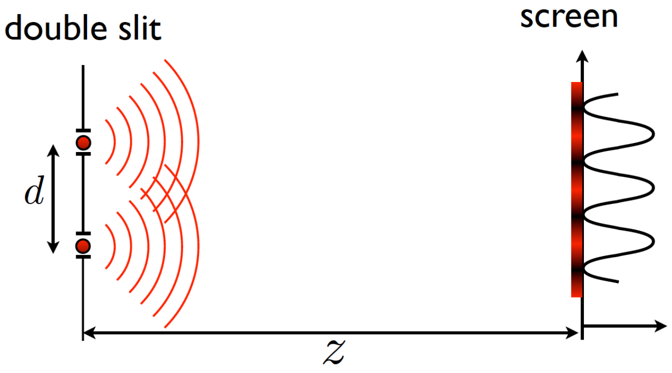


- Real, non-negative
- Models absorption and brightness

$$I_{tot} = I_1 + I_2$$

- Interpretation #2: Electromagnetic wave (*Coherent*)
- Model: Waves





- Complex field
- Models Interference

$$E_{tot} = E_1 + E_2$$

Alternative framework: Modeling coherent radiation as a wave