Tutorial 1. Training RU-Net

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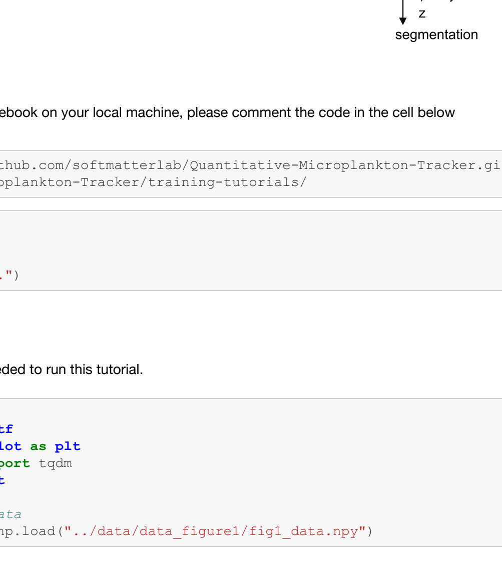
This tutorial demonstrates how to train a RU-Net (Regression-U-Net) for predicting plankton properties. It covers information on:

- Definining Simulation parameters
- Defining RU-Net using tensorflow keras
- Training RU-Net model
- Testing the model on an experimental images



NOTE:

- If you're running this notebook on your local machine, please comment the code in the cell below

```
In [4]: !git clone https://github.com/softmatterlab/Quantitative-Microplankton-Tracker.git
%cd Quantitative-Microplankton-Tracker/training-tutorials/
```

```
In [5]: %matplotlib inline
import os
import sys
sys.path.insert(0, "..")
```

1. Setup

Imports the dependecies needed to run this tutorial.

```
In [6]: import numpy as np
import tensorflow as tf
import matplotlib.pyplot as plt
from tqdm.notebook import tqdm
import deeptrack as dt

# Load experimental data
experimental_image = np.load("../data/data_figure1/fig1_data.npy")
```

2. Defining training dataset

2.1. Defining simulation parameters

We generate simulated holographic images of size 128 x 128 pixels to train the RU-Net. Each image contains plankton holograms of different properties as defined below.

```
In [7]: variables = {
    "pix_size": 3.6, # pixel size of the camera in microns - can be modified as per the camera size
    "radius_range": [1.5e-6, 10e-6], # range of the radius of the planktons in microns
    "ri_range": [1.35, 1.38], # range of the Refractive Index of the planktons
    "z_range": [2300, 3000], # z-distance of the plankton in microns (distance from the camera sensor)
    "apod_val": 0.07, #Gaussian apodization value (to account for the coherence of the source beam)
    "z_pixel": 20, # in microns (Ideally this should be the same as the pixel size) Used to reduce the
 memory usage
}
```

2.2. Defining scatterrer properties

Each plankton is a scatterrer. We use the `Sphere` feature of DeepTrack to generate spherical scatterrers. Since we are using lensless-holographic setup, planktons can be approximated as spherical objects. All the other properties are automatically filled from the variables defined above in the cell above.

```
In [8]: sphere_main = dt.Sphere(
    position=lambda: np.random.rand(2) * 128,
    position_unit="pixel",
    cam_pix_size=variables["pix_size"],
    radius=lambda: variables["radius_range"][0]
    + np.random.rand()
    * (variables["radius_range"][1] - variables["radius_range"][0]),
    refractive_index=lambda: variables["ri_range"][0]
    + np.random.rand() * (variables["ri_range"][1] - variables["ri_range"][0]),
    z=lambda: variables["z_range"][0] / variables["z_pixel"]
    + np.random.rand()
    * (variables["z_range"][1] - variables["z_range"][0])
    / variables["z_pixel"],
)
```

2.3. Defining the optical device

The scatterers are imaged with an optical device. We use the `Brightfield` feature of DeepTrack to define a microscope operating at `wavelength` of 633 nm.

```
In [9]: optics = dt.Brightfield(
    wavelength=633e-9,
    NA=1,
    #resolution=variables["pix_size"] * 1e-6,
    resolution=[variables["pix_size"] * 1e-6, variables["pix_size"] * 1e-6, variables["z_pixel"] * 1e-6
],
    magnification=1,
    refractive_index_medium=1.33,
    apod_val=variables["apod_val"],
    upscale=4,
    aberration=lambda apod_val: dt.GaussianApodization(apod_val + np.random.uniform(-1,1)*0.01),
    output_region=(0, 0, 128, 128),
)
```

2.4. Defining noises

We add gaussian noise to the generated images with a blur factor.

```
In [10]: from deeptrack.noises import Noise
from skimage.filters import gaussian
class Blurred_Gaussian(Noise):
    """Adds IID Gaussian noise to an image

    Parameters
    ----------
    mu : float
        The mean of the distribution.
    sigma : float
        The root of the variance of the distribution.
    """

    def __init__(self, mu=0, sigma=1,blur=2, **kwargs):
        super().__init__(mu=mu, sigma=sigma,blur=blur, **kwargs)

    def get(self, image, mu, sigma,blur, **kwargs):

        noisy_image = mu  + np.random.randn(*image.shape) * sigma
        noisy_image=gaussian(noisy_image,sigma=blur)
        noisy_image=image+noisy_image
        return noisy_image

noise = Blurred_Gaussian(mu=0, sigma= lambda: .025, blur=0.9+np.random.uniform(0,1)*0.1)
```

2.5. Defining number of planktons in 128 x 128 px image

Each image is defined to contain 5 to 8 number of planktons with randomly sampled properties.

```
In [11]: sphere_main_no = lambda: np.random.randint(5, 8)
sample_normal = sphere_main ** sphere_main_no
```

2.6. Combining all the properties defined above

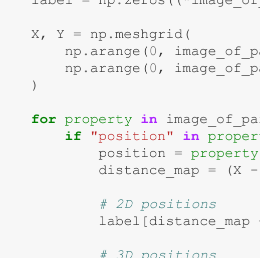
Passing all the features to the optical device to generate sample images.

```
In [12]: image_formed = optics(sample_normal)

image_of_particles = dt.ConditionalSetFeature(
    on_true=image_formed + noise,
    on_false=dt.FlipUD(dt.FlipLR(dt.FlipDiagonal(image_formed))) + noise,
    condition="skip_aug",
    skip_aug=False,
)
```

2.7. Visualising an example image

```
In [13]: simulated_image = image_of_particles.update(skip_aug=True).resolve()
plt.imshow(simulated_image[:,:,0], cmap="gray")
```

```
Out[13]: <matplotlib.image.AxesImage at 0x7fe3b7f1af10>
```



3. Creating target images

3.1. Dry mass values

Function to check the range of possible dry mass values for parameters defined above.

```
In [14]: def dm_range(rad_range, ri_range):
    m = lambda rad, ri: ((4 * np.pi) / 3) * ((rad * 1e6) ** 3) * (ri - 1.33)
    return m(rad_range[0], ri_range[0]), m(rad_range[1], ri_range[1])


dm_vals = dm_range(variables["radius_range"], variables["ri_range"])
dm_vals
```

```
Out[14]: (0.2827433388230816, 209.4395102393188)
```

3.2. Normalising target images

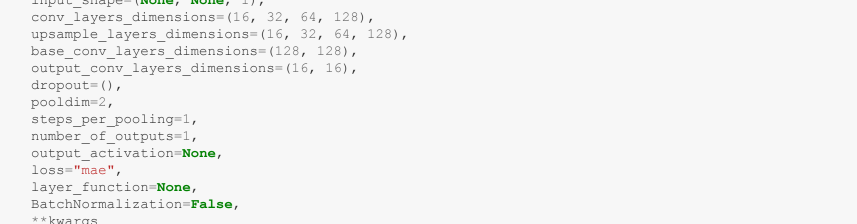
Function to generate the output channels for an input simulated image.

```
In [15]: def get_target_image(image_of_particles):
    label = np.zeros((*image_of_particles.shape[:2], 5))

    X, Y = np.meshgrid(
        np.arange(0, image_of_particles.shape[0]),
        np.arange(0, image_of_particles.shape[1]),
    )

    for property in image_of_particles.properties:
        if "position" in property:
            position = property["position"]
            distance_map = (X - position[1]) ** 2 + (Y - position[0]) ** 2

            # 2D positions
            label[distance_map < 9, 0] = 1

            # 3D positions
            z = property["z"]

            label[distance_map < 9, 1] = (
                z - variables["z_range"][0] / variables["z_pixel"]
            ) / (
                variables["z_range"][1] / variables["z_pixel"]
                - variables["z_range"][0] / variables["z_pixel"]
            )

            # Dry mass
            rad = property["radius"]
            ri = property["refractive_index"]
            dm = ((4 * np.pi) / 3) * ((rad * 1e6) ** 3) * (ri - 1.33)
            label[distance_map < 9, 2] = (dm - dm_vals[0]) / (
                dm_vals[1] - dm_vals[0]
            )

            label[distance_map < 9, 3] = (
                X[distance_map < 9] - position[1]
            ) / 3

            label[distance_map < 9, 4] = (
                Y[distance_map < 9] - position[0]
            ) / 3

    return label
```

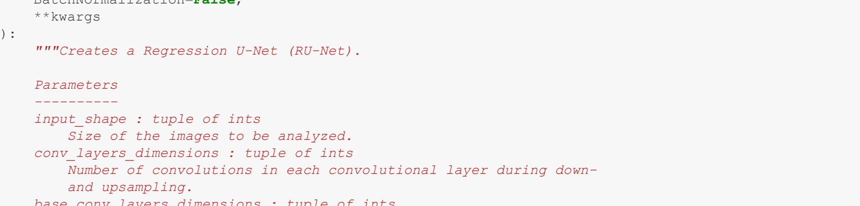
3.3. Visualising sample input and target images

```
In [16]: for i in range(3):
    image_of_particles.update(skip_aug=True)
    images = image_of_particles.resolve()
    label_of_particles = get_target_image(images)

    fig = plt.figure(figsize=(12*2, 9*2))

    print(np.shape(images))

    plt.subplot(1, 6,1)
    plt.imshow(images[..., 0], cmap="gray")

    plt.subplot(1,6,2)
    plt.title("background")
    plt.imshow(label_of_particles[..., 0])

    plt.subplot(1,6,3)
    plt.title("z")
    plt.imshow(label_of_particles[..., 1])

    plt.subplot(1,6,4)
    plt.title("dry mass")
    plt.imshow(label_of_particles[..., 2])

    plt.subplot(1,6,5)
    plt.title("x distance map")
    plt.imshow(label_of_particles[..., 3])

    plt.subplot(1,6,6)
    plt.title("y distance map")
    plt.imshow(label_of_particles[..., 4])

    plt.show()
```

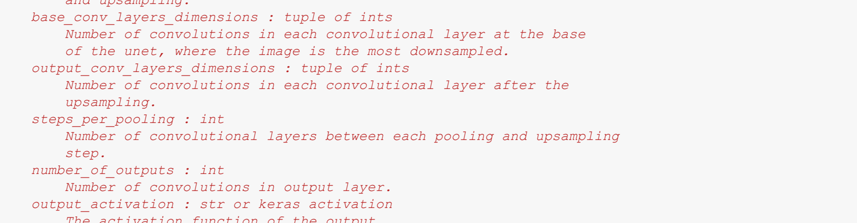
(128, 128, 1)



(128, 128, 1)



(128, 128, 1)



4. Training setup

4.1. Defining Regression U-Net using keras with tensorflow backend.

```
In [17]: def regressionUNet(
    input_shape=(None, None, 1),
    conv_layers_dimensions=(16, 32, 64, 128),
    upsample_layers_dimensions=(16, 32, 64, 128),
    base_conv_layers_dimensions=(128, 128),
    output_conv_layers_dimensions=(16, 16),
    dropout=(),
    pooldim=2,
    steps_per_pooling=1,
    number_of_outputs=1,
    output_activation=None,
    loss="mae",
    layer_function=None,
    BatchNormalization=False,
    **kwargs
):
    """Creates a Regression U-Net (RU-Net).

    Parameters
    ----------
    input_shape : tuple of ints
        Size of the images to be analyzed.
    conv_layers_dimensions : tuple of ints
        Number of convolutions in each convolutional layer during down-
        and upsampling.
    base_conv_layers_dimensions : tuple of ints
        Number of convolutions in each convolutional layer at the base
        of the unet, where the image is the most downsampled.
    output_conv_layers_dimensions : tuple of ints
        Number of convolutions in each convolutional layer after the
        upsampling.
    steps_per_pooling : int
        Number of convolutional layers between each pooling and upsampling
        step.
    number_of_outputs : int
        Number of convolutions in output layer.
    output_activation : str or keras activation
        The activation function of the output.
    loss : str or keras loss function
        The loss function of the network.
    layer_function : Callable[int] -> keras layer
        Function that returns a convolutional layer with convolutions
        determined by the input argument. Can be use to futher customize the network.

    Returns
    -------
    keras.models.Model
        Deep learning network.
    """

    def conv_step(layer, dimensions):
        layer = tf.keras.layers.Conv2D(
            dimensions, kernel_size=1, activation="relu", padding="same"
        )(layer)
        layern = tf.keras.layers.Conv2D(
            dimensions, kernel_size=3, activation="relu", padding="same"
        )(layer)

        layern = tf.keras.layers.Conv2D(
            dimensions, kernel_size=3, activation="relu", padding="same"
        )(layern)
        layer = tf.keras.layers.Add()([layer, layern])
        return layer

    if layer_function is None:
        layer_function = lambda dimensions: tf.keras.layers.Conv2D(
            conv_layer_dimension,
            kernel_size=3,
            activation="relu",
            padding="same",
        )

    unet_input = tf.keras.layers.Input(input_shape)

    concat_layers = []

    layer = unet_input

    # Downsampling step
    for conv_layer_dimension in conv_layers_dimensions:
        for _ in range(steps_per_pooling):
            layer = layer_function(conv_layer_dimension)(layer)
        concat_layers.append(layer)
        if BatchNormalization:
            layer = tf.keras.layers.BatchNormalization()(layer)
        if dropout:
            layer = tf.keras.layers.SpatialDropout2D(dropout[0])(layer)
            dropout = dropout[1:]

        layer = tf.keras.layers.MaxPooling2D(pooldim)(layer)

    # Base steps
    for conv_layer_dimension in base_conv_layers_dimensions:
        layer = layer_function(conv_layer_dimension)(layer)

    # Upsampling step
    regressionLayer = layer
    for conv_layer_dimension, concat_layer in zip(
        reversed(upsample_layers_dimensions), reversed(concat_layers)
    ):

        layer = tf.keras.layers.Conv2DTranspose(
            conv_layer_dimension, kernel_size=pooldim, strides=pooldim
        )(layer)
        regressionLayer = tf.keras.layers.Conv2DTranspose(
            conv_layer_dimension, kernel_size=pooldim, strides=pooldim
        )(regressionLayer)

        layer = tf.keras.layers.Concatenate(axis=-1)([layer, concat_layer])
        regressionLayer = tf.keras.layers.Concatenate(axis=-1)(
            [regressionLayer, concat_layer]
        )
        for _ in range(steps_per_pooling):
            layer = layer_function(conv_layer_dimension)(layer)
            regressionLayer = layer_function(conv_layer_dimension)(
                regressionLayer
            )

    # Output step
    for conv_layer_dimension in output_conv_layers_dimensions:
        layer = layer_function(conv_layer_dimension)(layer)
        regressionLayer = layer_function(conv_layer_dimension)(regressionLayer)
    layer = tf.keras.layers.Conv2D(
        1,
        kernel_size=3,
        activation="sigmoid",
        padding="same",
        name="segmentationOutput",
    )(layer)

    regressionLayer = tf.keras.layers.Concatenate(axis=-1)(
        [regressionLayer, layer]
    )
    regressionLayer = tf.keras.layers.Conv2D(
        number_of_outputs,
        kernel_size=3,
        activation=output_activation,
        padding="same",
        name="regressionOutput",
    )(regressionLayer)
    outputLayer = tf.keras.layers.Concatenate(axis=-1)(
        [layer, regressionLayer]
    )
    model = tf.keras.models.Model(inputs=unet_input, outputs=outputLayer)

    return model
```

4.2. Defining the loss function

We define a custom loss function as below for RU-Net.

```
In [18]: import tensorflow.keras.backend as K

eps = 1e-6


def softmax_categorical(T, P):
    classwise_weight = K.mean(1 - T, axis=(1, 2), keepdims=True)
    true_error = K.mean(T * K.log(P + eps) * classwise_weight, axis=-1)
    return -K.mean(true_error)


def unet_features(weight=(10, 1, 0.1), num_features=1):
    def get_loss(y_true, y_pred):
        T = K.flatten(y_true[:, :, :, 0])
        P = K.flatten(y_pred[:, :, :, 0])

        error = K.abs(T - P)
        loss = -K.mean(
            weight[0] * T * K.log(P + 1e-4)
            + weight[1] * (1 - T) * K.log(1 - P + 1e-4)
        ) / (
            weight[0] + weight[1]
        )
        for i in range(num_features):

            T1 = K.flatten(y_true[:, :, :, i + 1])
            P1 = K.flatten(y_pred[:, :, :, i + 1])
            error = K.abs(T1 - P1)
            f_loss = K.sum(T * error) / (K.sum(T) + 1e-6) + K.sum(
                error * (1 - T)
            ) / (K.sum(1 - T) + 1e-6)
            loss += weight[2] * f_loss

        return loss

    return get_loss
```

4.3. Defining the model parameters

```
In [19]: model = regressionUNet(
    (None, None, 1),
    conv_layers_dimensions=[8, 16, 32, 64],
    base_conv_layers_dimensions=[32, 32],
    number_of_outputs=4,
    output_conv_layers_dimensions=[32, 32],
    loss=unet_features(num_features=4, weight=(1, 1, 1)),
    output_activation=None,
)

model.summary()
```

```
Model: "functional_1"
__________________________________________________________________________________________________
Layer (type)                    Output Shape         Param #     Connected to
==================================================================================================
input_1 (InputLayer)            [(None, None, None,  0
__________________________________________________________________________________________________
conv2d (Conv2D)                 (None, None, None, 8 80          input_1[0][0]
__________________________________________________________________________________________________
max_pooling2d (MaxPooling2D)    (None, None, None, 8 0           conv2d[0][0]
__________________________________________________________________________________________________
conv2d_1 (Conv2D)               (None, None, None, 1 1168        max_pooling2d[0][0]
__________________________________________________________________________________________________
max_pooling2d_1 (MaxPooling2D)  (None, None, None, 1 0           conv2d_1[0][0]
__________________________________________________________________________________________________
conv2d_2 (Conv2D)               (None, None, None, 3 4640        max_pooling2d_1[0][0]
__________________________________________________________________________________________________
max_pooling2d_2 (MaxPooling2D)  (None, None, None, 3 0           conv2d_2[0][0]
__________________________________________________________________________________________________
conv2d_3 (Conv2D)               (None, None, None, 6 18496       max_pooling2d_2[0][0]
__________________________________________________________________________________________________
max_pooling2d_3 (MaxPooling2D)  (None, None, None, 6 0           conv2d_3[0][0]
__________________________________________________________________________________________________
conv2d_4 (Conv2D)               (None, None, None, 3 18464       max_pooling2d_3[0][0]
__________________________________________________________________________________________________
conv2d_5 (Conv2D)               (None, None, None, 3 9248        conv2d_4[0][0]
__________________________________________________________________________________________________
conv2d_transpose (Conv2DTranspo (None, None, None, 1 16512       conv2d_5[0][0]
__________________________________________________________________________________________________
concatenate (Concatenate)       (None, None, None, 1 0           conv2d_transpose[0][0]
                                                                 conv2d_3[0][0]
__________________________________________________________________________________________________
conv2d_transpose_1 (Conv2DTrans (None, None, None, 1 16512       conv2d_5[0][0]
__________________________________________________________________________________________________
conv2d_6 (Conv2D)               (None, None, None, 1 221312      concatenate[0][0]
__________________________________________________________________________________________________
concatenate_1 (Concatenate)     (None, None, None, 1 0           conv2d_transpose_1[0][0]
                                                                 conv2d_3[0][0]
__________________________________________________________________________________________________
conv2d_transpose_2 (Conv2DTrans (None, None, None, 6 32832       conv2d_6[0][0]
__________________________________________________________________________________________________
conv2d_7 (Conv2D)               (None, None, None, 1 221312      concatenate_1[0][0]
__________________________________________________________________________________________________
concatenate_2 (Concatenate)     (None, None, None, 9 0           conv2d_transpose_2[0][0]
                                                                 conv2d_2[0][0]
__________________________________________________________________________________________________
conv2d_transpose_3 (Conv2DTrans (None, None, None, 6 32832       conv2d_7[0][0]
__________________________________________________________________________________________________
conv2d_8 (Conv2D)               (None, None, None, 6 55360       concatenate_2[0][0]
__________________________________________________________________________________________________
concatenate_3 (Concatenate)     (None, None, None, 9 0           conv2d_transpose_3[0][0]
                                                                 conv2d_2[0][0]
__________________________________________________________________________________________________
conv2d_transpose_4 (Conv2DTrans (None, None, None, 3 8224        conv2d_8[0][0]
__________________________________________________________________________________________________
conv2d_9 (Conv2D)               (None, None, None, 6 55360       concatenate_3[0][0]
__________________________________________________________________________________________________
concatenate_4 (Concatenate)     (None, None, None, 4 0           conv2d_transpose_4[0][0]
                                                                 conv2d_1[0][0]
__________________________________________________________________________________________________
conv2d_transpose_5 (Conv2DTrans (None, None, None, 3 8224        conv2d_9[0][0]
__________________________________________________________________________________________________
conv2d_10 (Conv2D)              (None, None, None, 3 13856       concatenate_4[0][0]
__________________________________________________________________________________________________
concatenate_5 (Concatenate)     (None, None, None, 4 0           conv2d_transpose_5[0][0]
                                                                 conv2d_1[0][0]
__________________________________________________________________________________________________
conv2d_transpose_6 (Conv2DTrans (None, None, None, 1 2064        conv2d_10[0][0]
__________________________________________________________________________________________________
conv2d_11 (Conv2D)              (None, None, None, 3 13856       concatenate_5[0][0]
__________________________________________________________________________________________________
concatenate_6 (Concatenate)     (None, None, None, 2 0           conv2d_transpose_6[0][0]
                                                                 conv2d[0][0]
__________________________________________________________________________________________________
conv2d_transpose_7 (Conv2DTrans (None, None, None, 1 2064        conv2d_11[0][0]
__________________________________________________________________________________________________
conv2d_12 (Conv2D)              (None, None, None, 1 3472        concatenate_6[0][0]
__________________________________________________________________________________________________
concatenate_7 (Concatenate)     (None, None, None, 2 0           conv2d_transpose_7[0][0]
                                                                 conv2d[0][0]
__________________________________________________________________________________________________
conv2d_14 (Conv2D)              (None, None, None, 3 4640        conv2d_12[0][0]
__________________________________________________________________________________________________
conv2d_13 (Conv2D)              (None, None, None, 1 3472        concatenate_7[0][0]
__________________________________________________________________________________________________
conv2d_16 (Conv2D)              (None, None, None, 3 9248        conv2d_14[0][0]
__________________________________________________________________________________________________
conv2d_15 (Conv2D)              (None, None, None, 3 4640        conv2d_13[0][0]
__________________________________________________________________________________________________
segmentationOutput (Conv2D)     (None, None, None, 1 289         conv2d_16[0][0]
__________________________________________________________________________________________________
conv2d_17 (Conv2D)              (None, None, None, 3 9248        conv2d_15[0][0]
__________________________________________________________________________________________________
concatenate_8 (Concatenate)     (None, None, None, 3 0           conv2d_17[0][0]
                                                                 segmentationOutput[0][0]
__________________________________________________________________________________________________
regressionOutput (Conv2D)       (None, None, None, 4 1192        concatenate_8[0][0]
__________________________________________________________________________________________________
concatenate_9 (Concatenate)     (None, None, None, 5 0           segmentationOutput[0][0]
                                                                 regressionOutput[0][0]
==================================================================================================
Total params: 788,617
Trainable params: 788,617
Non-trainable params: 0
__________________________________________________________________________________________________
```

```
In [20]: model.compile(
    tf.keras.optimizers.Adam(lr=0.0001, amsgrad=True),
    loss=unet_features(num_features=4, weight=(1, 1, 1)),
)
```

4.4. Defining batch function

```
In [21]: def batch_function(image):
    return image / np.median(image)
```

4.5. Define generator

The generator generates `5000` images before the training begins, and continous to generate another `5000` during the training process

```
In [22]: generator = dt.generators.ContinuousGenerator(
    image_of_particles,
    get_target_image,
    batch_size=64,
    batch_function=batch_function,
    min_data_size=5000,
    max_data_size=10000,
)
```

5. Training the model

Set the `TRAIN_MODEL` = `True` to train the model from scratch. Set the `TRAIN_MODEL` = `False` to load a pretrained model.

```
In [23]: TRAIN_MODEL = False
```

5.1. Generating validation data

Skip this step if would not like to generate validation data

```
In [24]: if TRAIN_MODEL:

    print("Generating validation data..")
    b = []
    l = []
    for i in tqdm(range(1000)):
        im = image_of_particles.update().resolve()
        b.append(batch_function(im))
        l.append(get_target_image(im))
    b = np.array(b)
    l = np.array(l)
```

5.2. Start the training

Set the `TRAIN_MODEL` to True to train the network from scratch

```
In [25]: if TRAIN_MODEL:
    with generator:
        history = model.fit(
            generator,
            epochs=1000,
            steps_per_epoch=16,
            #validation_data=(b, l),
            max_queue_size=0,
            workers=0,
        )

else:

    model.load_weights("../pre-trained-models/RUNet.h5")
```

6. Testing the trained model on experimental image

6.1. Normalising the experimental image

```
In [26]: img = experimental_image/np.median(experimental_image)
img = img.reshape([1,1024, 1280, 1])
predicted_image = model.predict(img)
```

6.2. Checking predictions

In [27]:
```python
#predictions
fig=plt.figure(figsize=(50,50))

[a,b,c,d]=[680,880,375,575]

plt.subplot(6,1,1)
plt.imshow((experimental_image/np.median(experimental_image))[a:b, c:d] , cmap="gray")
plt.title("Original")
plt.colorbar()

plt.subplot(6,1,2)
plt.imshow(np.squeeze(predicted_image[:, :, :, 0]) [a:b, c:d])
plt.title("Predicted, background")
plt.colorbar()

plt.subplot(6,1,3)
plt.imshow(np.squeeze(predicted_image[:, :, :, 1]) [a:b, c:d])
plt.title("Predicted, axial distance")
plt.colorbar()

plt.subplot(6,1,4)
plt.imshow(np.squeeze(predicted_image[:, :, :, 2]) [a:b, c:d])
plt.title("Predicted, dry mass")
plt.colorbar()

plt.subplot(6,1,5)
plt.imshow(np.squeeze(predicted_image[:, :, :, 3]) [a:b, c:d])
plt.title("Predicted, delta x")
plt.colorbar()

plt.subplot(6,1,6)
plt.imshow(np.squeeze(predicted_image[:, :, :, 4]) [a:b, c:d])
plt.title("Predicted, delta y")
plt.colorbar()

plt.show()
```