Example 3. Analysis pipeline (Using RU-Net and WAC-Net in sequence)

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This example demonstrates how to use a pre-trained RU-Net and WAC-Net in sequence to get the dry mass values

NOTE:

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

In [1]:
```
!git clone https://github.com/softmatterlab/Quantitative-Microplankton-Tracker.git
%cd Quantitative-Microplankton-Tracker/examples/
```

In [2]:
```
%matplotlib inline
import sys
sys.path.append("..")
```

1. Setup

Imports the needed dependencies to run this tutorial.

In [3]:
```
import numpy as np
import pandas as pd
import tensorflow as tf
import matplotlib as mpl
import matplotlib.pyplot as plt
from tqdm.notebook import tqdm
from skimage.measure import label, regionprops
import deeptrack as dt
```

2. Loading experimental holographic image

We use a 50 frame experimental image sequence containing species in this tutorial. Experimental images are located in **sample-data** folder.

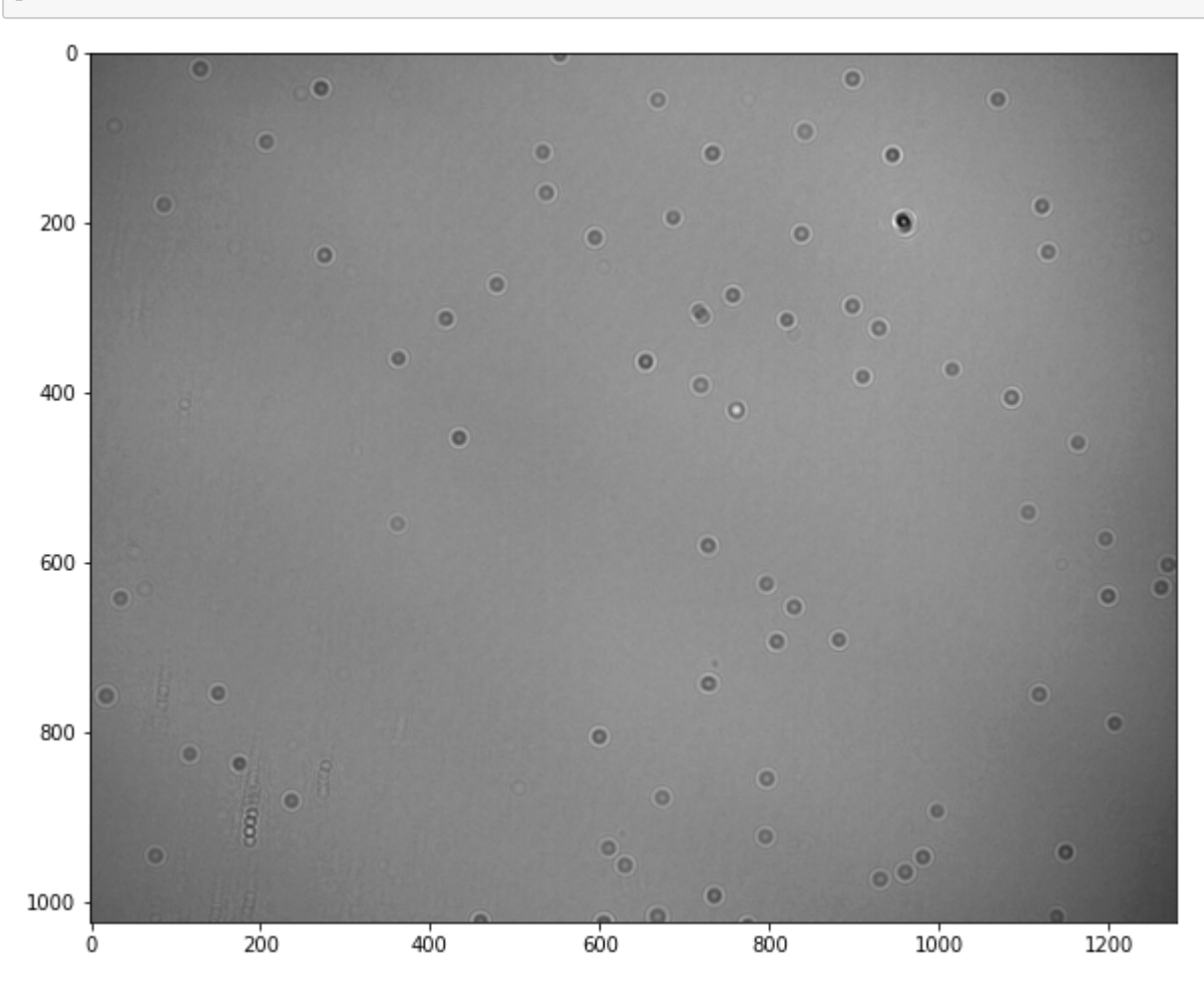
In [4]:
```
exp_data = np.load(
    "../data/data_figure2/Oxyrrhis_data.npy"
)
np.shape(exp_data)
```

Out[4]: (50, 1024, 1280)

2.1. Visualising experimental image

First frame of the experimental holographic sequence.

In [5]:
```
plt.figure(figsize=(12, 8))
plt.imshow(exp_data[0], cmap="gray")
plt.show()
```



3. Using RU-Net on experimental images

We use RU-Net model to segment planktons from the background, and to extract their properties. Pre-trained models are located in `pre-trained models` folder

3.1. Load pre-trained RU-Net model

In [6]:
```
RUNet_model = tf.keras.models.load_model(
    "../pre-trained-models/RUNet.h5",
    compile=False
)
```

3.2. Predictions followed by extracting properties

`predict_image` function extracts the 2d positions, dry mass and z-distance from predictions of RU-Net

In [7]:
```
def predict_image(predicted_image, threshold):
    """
    Outputs the predicted channels for regression U-nets
    Inputs: image and image threshold, Eg., 0.1
    Outputs: xpositions, ypositions, zpositions, drymass, and eccentricity
    """
    Data = []

    X, Y = np.meshgrid(
        range(0, predicted_image.shape[2]), range(0, predicted_image.shape[1])
    )
    X = np.reshape(X, (predicted_image.shape[1] * predicted_image.shape[2]))
    Y = np.reshape(Y, (predicted_image.shape[1] * predicted_image.shape[2]))

    for i in range(predicted_image.shape[0]):

        zpos = []
        mass = []
        xpos = []
        ypos = []
        ecc = []
        xyri = []

        predX = predicted_image[i, :, :, 3]
        predY = predicted_image[i, :, :, 4]

        gx1, gy1 = np.gradient(predX)
        gx2, gy2 = np.gradient(predY)

        I = gy1 + gx2

        I2 = np.zeros(I.shape)
        I2[I > threshold] = 1

        I3 = label(I2)
        prop = regionprops(I3)

        zs = predicted_image[i, :, :, 1]
        masses = predicted_image[i, :, :, 2]
        I3 = np.reshape(
            I3, (predicted_image.shape[2] * predicted_image.shape[1])
        )
        zs = np.reshape(
            zs, (predicted_image.shape[2] * predicted_image.shape[1])
        )
        masses = np.reshape(
            masses, (predicted_image.shape[2] * predicted_image.shape[1])
        )

        for j in range(np.amax(I3)):
            if prop[j].eccentricity < 1.2:

                ecc.append(prop[j].eccentricity)

                inds = np.where(I3 == j + 1)
                zpos.append(np.mean(zs[inds]))
                mass.append(np.mean(masses[inds]))
                xpos.append(np.mean(X[inds]))
                ypos.append(np.mean(Y[inds]))

                xyri.append(
                    [
                        np.mean(X[inds]),
                        np.mean(Y[inds]),
                        np.mean(zs[inds]),
                        np.mean(masses[inds]),
                    ]
                )

        xyri = np.array(xyri)
        Data.append(xyri)

    return Data
```

3.3. Preparing the images for prediction

Split the 50 frame sequence into batches of 5 for prediction

In [8]:
```
batchsize = 5
data_in_batches = [
    exp_data[i : i + batchsize] for i in range(0, int(len(exp_data)), batchsize)
]
```

Function to obtain RU-Net predictions on a batch of images

In [9]:
```
def get_prediction_batch(batch, model):
    '''
    return the prediction of a batch of images
    '''
    batch_reshape = np.expand_dims(batch, axis=3)
    prediction = model.predict(batch_reshape)
    return prediction
```

3.4. Predicting positions

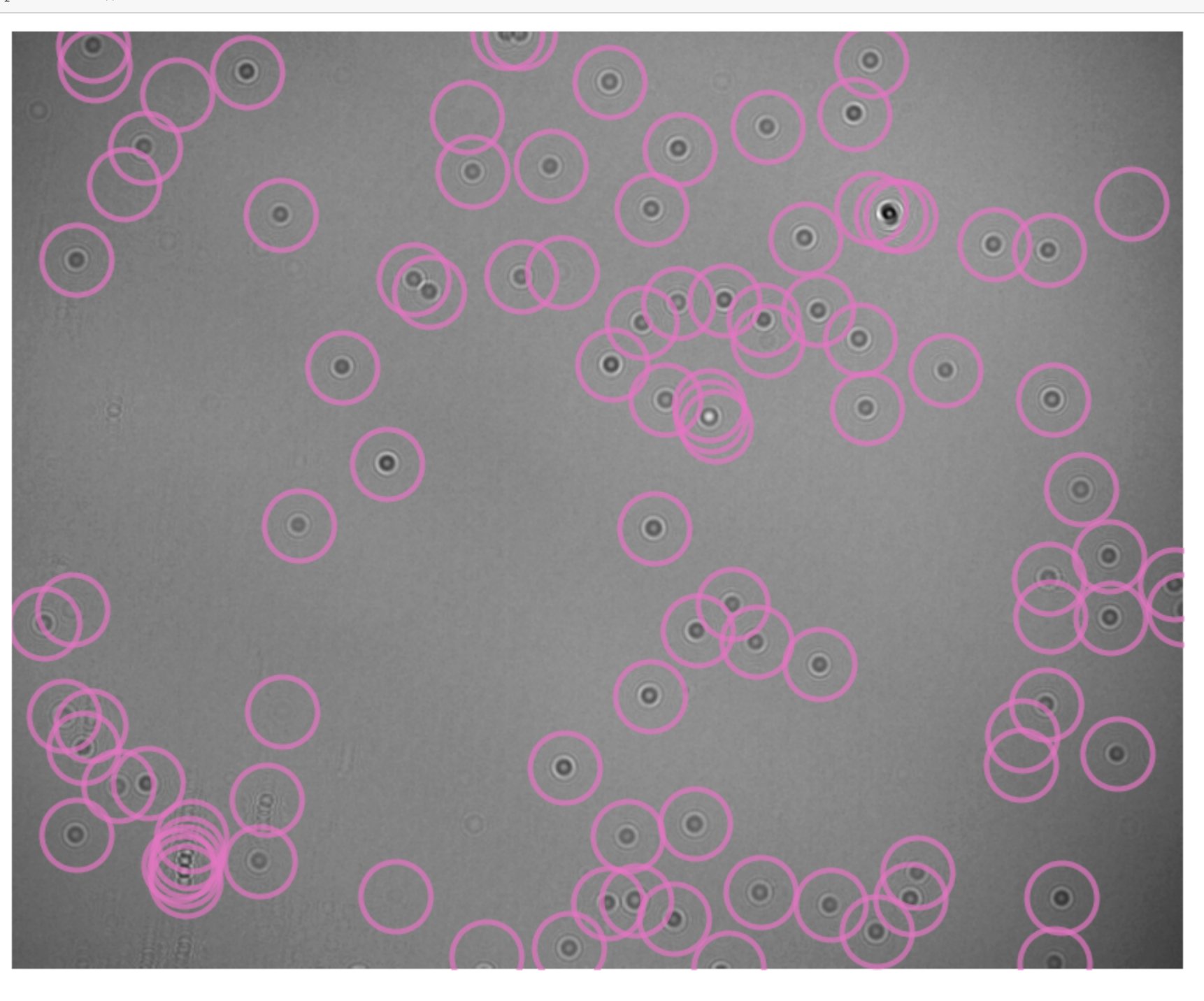
Get the positions of planktons from the RU-Net predictions. The positions are stored in `position_data`. This process should take about a minute.

In [10]:
```
position_data = []

threshold = 0.1
median_val = np.median(exp_data[0])

for i in tqdm(range(len(data_in_batches)), desc="Predicting batch "):
    batch = data_in_batches[i]

    prediction = get_prediction_batch(
        batch / median_val,
        RUNet_model
    )

    positions = predict_image(
        prediction,
        threshold
    )

    for coords in positions:
        position_data.append(coords)
```

3.5. Highlighting the plankton positions

Change `frame_no` to higlight plankton positions in other frames

In [11]:
```
frame_no = 10

fig, ax = plt.subplots(figsize=(15,15))

ax.imshow(exp_data[frame_no], cmap='gray')

X = position_data[frame_no][:, 0]
Y = position_data[frame_no][:, 1]

for i in range(0,len(X),1):
    x0 = X[i]
    y0 = Y[i]
    ax.plot(x0, y0,'o', ms=50, markerfacecolor="None", markeredgecolor="#e377c2", markeredgewidth=4, al
pha=0.7)

plt.axis("off")
plt.show()
```



4. Plankton trajectories

4.1. Getting plankton traces

We use `get_particle_trace` function to link the plankton positions in all the frames to individual trajectories.

In [12]:
```
particles, completed_traces = dt.get_particle_trace(position_data)
print('Particles', np.shape(particles), 'completed traces', np.shape(completed_traces))
all_traces = completed_traces + particles[0]
print("Number of particle traces ", len(all_traces))
```

```
Particles (1, 110) completed traces (30,)
Number of particle traces  140
```

4.2. Formatting trajectories into a dataframe

Formatting trajectories into dataframes makes it easy to read the trajectories

In [13]:
```
list_of_dataframes = []

for i in range(len(all_traces)):

    df = pd.DataFrame(all_traces[i], columns = ['frame', 'x', 'y', 'z', 'drymass'])
    df = df.assign(particle = [i]*len(all_traces[i]))

    list_of_dataframes.append(df)

particle_data = pd.concat(list_of_dataframes, ignore_index = True)

particle_data = particle_data[particle_data.x != 0] #deletes all rows where x=0 and y=0, because that's
 probably some weird prediction at the edge
particle_data = particle_data[particle_data.y != 0]

particle_data
```

Out[13]:

	frame	x	y	z	drymass	particle
0	0	1145.125000	601.875000	-0.054241	0.187524	0
1	1	1144.833333	601.833333	-0.081731	0.178887	0
2	2	1145.000000	602.285714	-0.081766	0.184561	0
3	3	1145.500000	601.500000	-0.061431	0.125581	0
4	4	1145.333333	602.333333	-0.109081	0.147072	0
...	...	...	...	...	...	...
4201	49	1043.500000	783.000000	0.079965	0.048296	135
4202	49	230.000000	838.500000	0.121690	0.077743	136
4203	49	10.200000	861.400000	0.190252	0.102897	137
4204	49	565.000000	1013.250000	0.169295	0.102271	138
4205	49	767.000000	1023.000000	-0.015778	0.117703	139

4206 rows × 6 columns

4.3. Processing trajectories

Trajectories are processed and relevant information is stored in `traj_data` array.

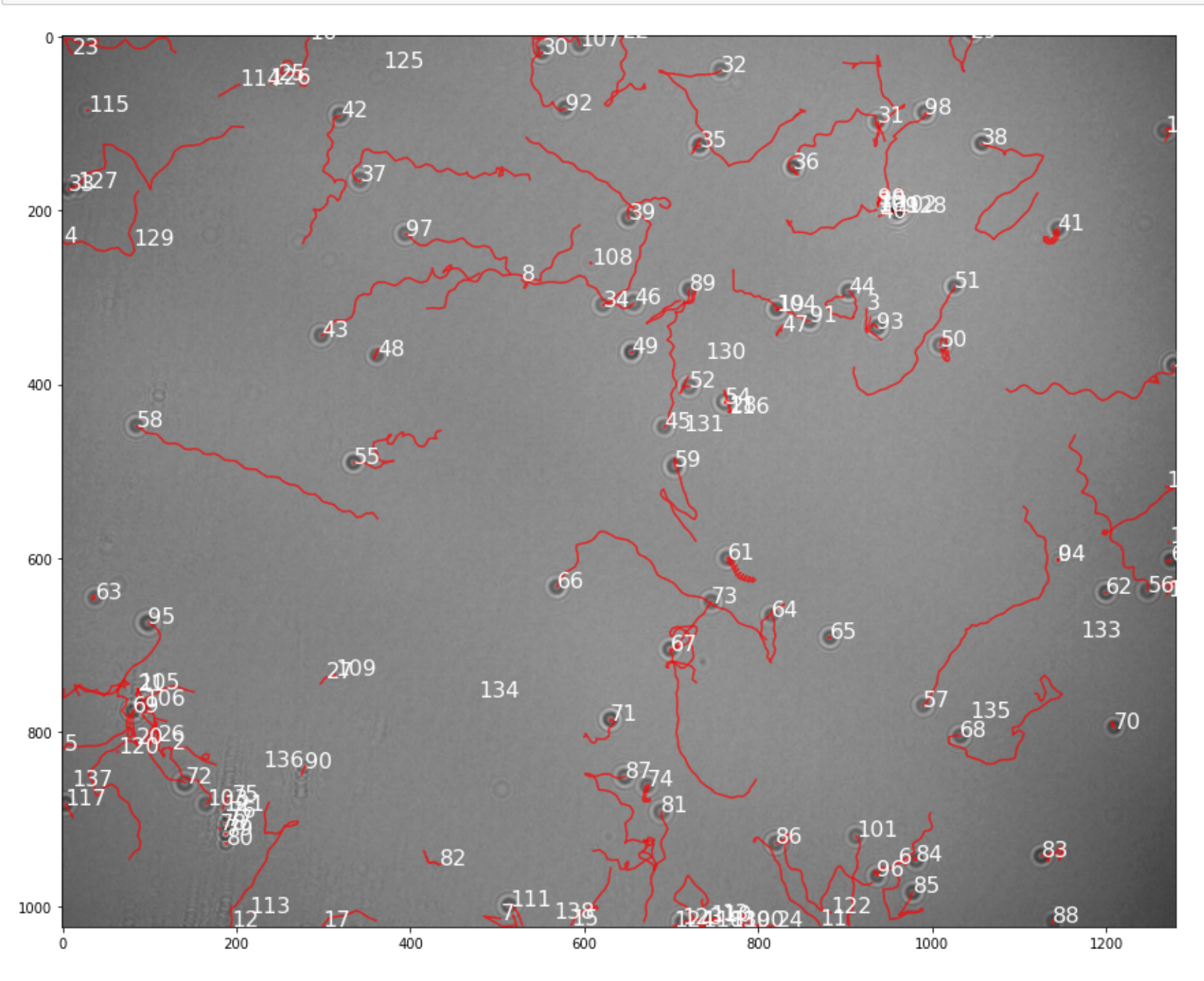
In [14]:
```
trajs = []
trajs_lengths = []

positions=particle_data.set_index(['particle', 'frame'])[['x', 'y']].unstack()

for index, rows in positions.iterrows():
    traj_len=np.count_nonzero(~np.isnan(rows[['x', 'y'][0]]))

    trajs_lengths.append(traj_len)
    trajs.append(np.transpose([rows[['x','y'][0]].to_numpy(), rows[['x', 'y'][1]].to_numpy()]))
```

In [15]:
```
# Appending the frame number before the nodes
traj_data = []
for i in range(len(trajs)):
    non_nan_indices = np.where(np.isnan(trajs[i][:,0]) == False)[0]
    frame_no = np.reshape(non_nan_indices, (len(non_nan_indices),1))
    frame_and_centroids = np.append(frame_no, trajs[i][non_nan_indices], axis=1)
    traj_data.append(frame_and_centroids)
```

4.4. Visualising the trajectories

Trajectories are plotted over the final frame of the sequence for easy visualization. The index of the trajectory helps to isloate individual single plankton traces.

In [16]:
```
fig, ax=plt.subplots(figsize=(18, 12))
ax.imshow(exp_data[-1], cmap="gray")
i = 0
for trajectory in traj_data:
    ax.plot(trajectory[:,1], trajectory[:,2], "red", alpha=0.7, linewidth=1.5)
    ax.text(trajectory[:,1][-1], trajectory[:,2][-1], str(i), color="white", fontsize=16)
    i += 1
```



5. Dry mass prediction by WAC-Net

We now crop 64 x 64 pixel images around the single plankton traces, and predict a improved dry mass values with a pre-trained WAC-Net.

5.1. Selecting planktons

Pick a plankton trace by their `number` in the above figure for the dry mass prediction. Alternatively, dry mass can also be predicted for all the planktons

In [17]:
```
trace_no = 58
```

Function to obtain the crop around the plankton position

In [18]:
```
def croppedimage(image, position, window=32):
    """
    crops a region of 2*window pixels around the position
    """
    [a, b, c, d] = [
        int(position[0] - window),
        int(position[0] + window),
        int(position[1] - window),
        int(position[1] + window),
    ]
    return image[c:d, a:b]
```

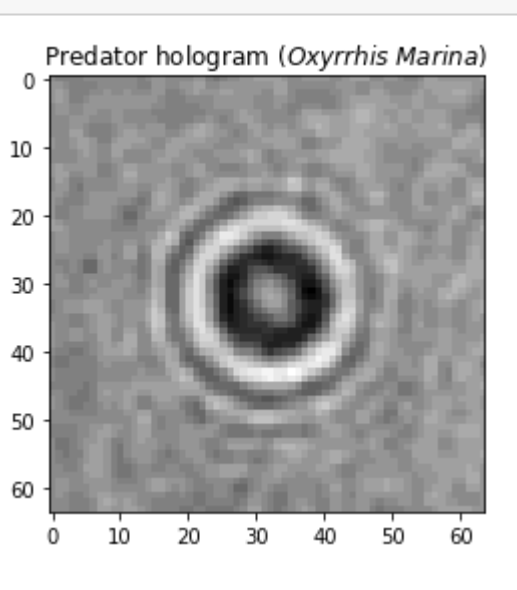
5.2. Obtaining sequence of crops for a single plankton trajectory

Crops are stored in `cropped_sequence`

In [19]:
```
cropped_sequence = []
single_plankton_traj = traj_data[trace_no]

for i in range(len(single_plankton_traj)):

    crop = croppedimage(
        exp_data[
            int(single_plankton_traj[i][0])
            ],
        single_plankton_traj[i][1:3]
    )

    cropped_sequence.append(crop)
```

Visuazlise first frame in the cropped plankton sequence

In [20]:
```
plt.imshow(cropped_sequence[0], cmap="gray")
plt.title("Predator hologram ($\it{Oxyrrhis\ Marina}$)")
plt.show()
```



5.3. Loading pre-trained WAC-Net model for dry mass model

We define the WAC-Net model and load the weights from a pre-trained model.

In [21]:
```
from tensorflow import keras

Sequential = keras.models.Sequential
Model = keras.models.Model
Dense = keras.layers.Dense
Conv = keras.layers.Conv2D
Conv1D = keras.layers.Conv1D
ConvL = keras.layers.LocallyConnected2D
Pool = keras.layers.MaxPooling2D
Input = keras.layers.Input
Concat = keras.layers.Concatenate
TimeDistributed = keras.layers.TimeDistributed
Flatten = keras.layers.Flatten
Lambda = keras.layers.Lambda
K = keras.backend


model = Sequential()
model.add(
    (
        Conv(
            32,
            kernel_size=3,
            strides=1,
            activation="relu",
            input_shape=(64, 64, 1),
        )
    )
)
model.add(Pool(2))
model.add((Conv(64, kernel_size=3, strides=1, activation="relu")))
model.add(Pool(2))
model.add((Conv(128, kernel_size=3, strides=1, activation="relu")))
model.add(Pool(2))
model.add((Conv(256, kernel_size=3, strides=1, activation="relu")))
model.add((Flatten()))
model.add((Dense(128, activation="relu")))
model.add((Dense(128, activation="relu")))

stack = Input(model.input_shape)
vectors = TimeDistributed(model)(
    stack
)  # time distributed applies a layer to every temporal slice of the input
weights = Conv1D(128, 1, padding="same")(vectors)
weights = Conv1D(128, 1, padding="same")(weights)
weights = Conv1D(1, 1, padding="same")(weights)


def merge_function(tensors):
    x = tensors[0]
    weights = tensors[1]
    weights = K.softmax(weights, axis=1)
    merged = K.sum(x * weights, axis=1)
    return merged


merge_layer = Lambda(merge_function)

merged = merge_layer([vectors, weights])

merged = Dense(32, activation="relu")(merged)
merged = Dense(32, activation="relu")(merged)
out = Dense(2)(merged)
model = Model(stack, out)

model.compile(tf.keras.optimizers.Adam(lr=0.0001, amsgrad=True), loss="mae")
model.load_weights("../pre-trained-models/WACNet_dry_mass.h5")
```

5.4. Normalising the crops

Normalise and reshape the images for WAC-Net prediction

In [22]:
```
def Normalise(images, batch = 15):
    Normalised = []
    for i in range(len(images)):
        Normalised.append(images[i]/np.median(images[i]))
    Normalised = np.array(Normalised)-1
    proc = []

    #sliding window
    for i in range(len(Normalised)-batch+1):
        proc.append(Normalised[i:i+batch])
    return np.expand_dims(proc, axis = -1)
```

In [23]:
```
# Funtion to convert the predicted dry mass to real dry mass units (pico grams)
def real_dm(p, a=209.16, b=0.28, sp_ri_inc = 0.21):
    return (p*a+b)/sp_ri_inc
```

5.5. Prediction on cropped sequences

Experimental sequences are normalized with `batch` size of 15. `batch` value can be increased or decreased to generate a sliding window of the experimental sequence. The WAC-Net assigns weights to the images with in sliding windows to predict the best possible value for dry mass considering all the frames in a sliding window.

In [25]:
```
prediction = model.predict(
    Normalise(
        cropped_sequence,
        batch = 15
    )
)
```

5.6. Convert the predictions to real dry mass units

In [26]:
```
drymass_prediction = real_dm(prediction[:,0])
```

5.7. Visualising dry mass predictions

In [27]:

```
plt.title("Refined dry mass prediction by WAC-Net")
plt.plot(drymass_prediction, "red")
plt.xlabel('Time (seconds)')
plt.ylabel('Drymass (in picograms)')
xticks = np.arange(0,550,50)
ax.set_xticks(xticks)
ax.set_xticklabels([int(x*(1/10)) for x in xticks])
plt.show()
```