Example 2. Using WAC-Net

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This example demonstrates how to use a pre-trained WAC-Net (Weighted average convolutional neural network) on experimental images to extract more accurate values of dry mass

NOTE:

- If you're using google colaboratory to run this notebook, please uncomment the code in the following cell to clone the repository.
- If you're running the notebook on your local machine, please skip this step to avoid cloing the repository in the current folder.

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

Import the dependencies to run this tutorial.

In [3]:
```
import glob
import numpy as np
import tensorflow as tf
import matplotlib.pyplot as plt
import deeptrack as dt
```

2. Load experimental holographic image

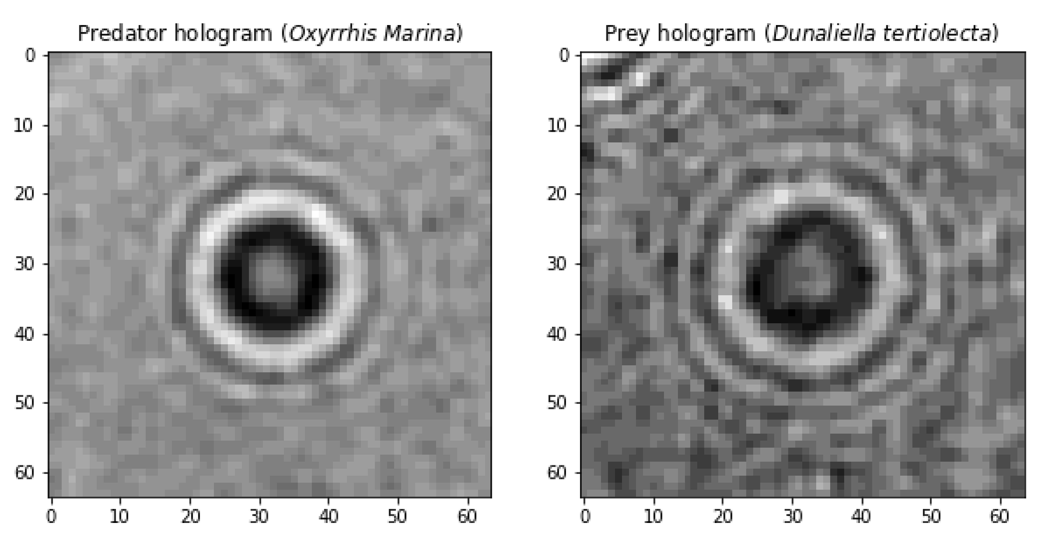
Experimental images are located in `sample-data` folder

In [4]:
```
# Load figure 3 data (feeding events)
predator_sequence = np.load(
    "../data/data_figure3/predator_sequence.npy"
)

prey_sequence = np.load(
    "../data/data_figure3/prey_sequence.npy"
)

# Load figure 4 data (division events)
cell1_sequence = np.load(
    "../data/data_figure4/Cell1_sequence.npy"
)

cell2_sequence = np.load(
    "../data/data_figure4/Cell2_sequence.npy"
)

cell3_sequence = np.load(
    "../data/data_figure4/Cell3_sequence.npy"
)
```

2.1. Visualize prey and predator planktons

In [5]:
```
plt.figure(figsize=(10, 5))
plt.subplot(1,2,1)
plt.title("Predator hologram ($\it{Oxyrrhis\ Marina}$)")
plt.imshow(predator_sequence[0], cmap="gray")

plt.subplot(1,2,2)
plt.title("Prey hologram ($\it{Dunaliella\ tertiolecta}$)")
plt.imshow(prey_sequence[0], cmap="gray")
```

Out[5]:
```
<matplotlib.image.AxesImage at 0x7fa5a68ef6a0>
```



3. Load pre-trained WAC-Net model

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

In [6]:
```
# WAC-Net trained for feeding events

feeding_model = dt.models.WACNet(outputs = 2) # 2 outputs: dry mass and radius
feeding_model.load_weights(
    "../pre-trained-models/WACNet_dry_mass.h5"
)

# WAC-Net models trained for division events

division_event_models_loc = glob.glob(
    "../pre-trained-models/division-event-models/*.h5"
)

division_models = []
for model_loc in division_event_models_loc:
    empty_model = dt.models.WACNet(outputs = 1)
    empty_model.load_weights(model_loc)
    division_models.append(empty_model)
```

4. WAC-Net prediction on experimental sequences

4.1. Example 1 - Feeding events

Feeding events are recorded in two sequences:

- `predator_sequence` contains predator frames
- `prey_sequence` contains prey frames

4.1.1. Normalise the experimental image

Normalise and reshape the image for WAC-Net prediction

In [7]:
```
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 [8]:
```
# 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
```

4.1.2. Prediction on experimental sequences

Experimental sequences are normalized with `batch` size of 1. `batch` value can be increased 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.

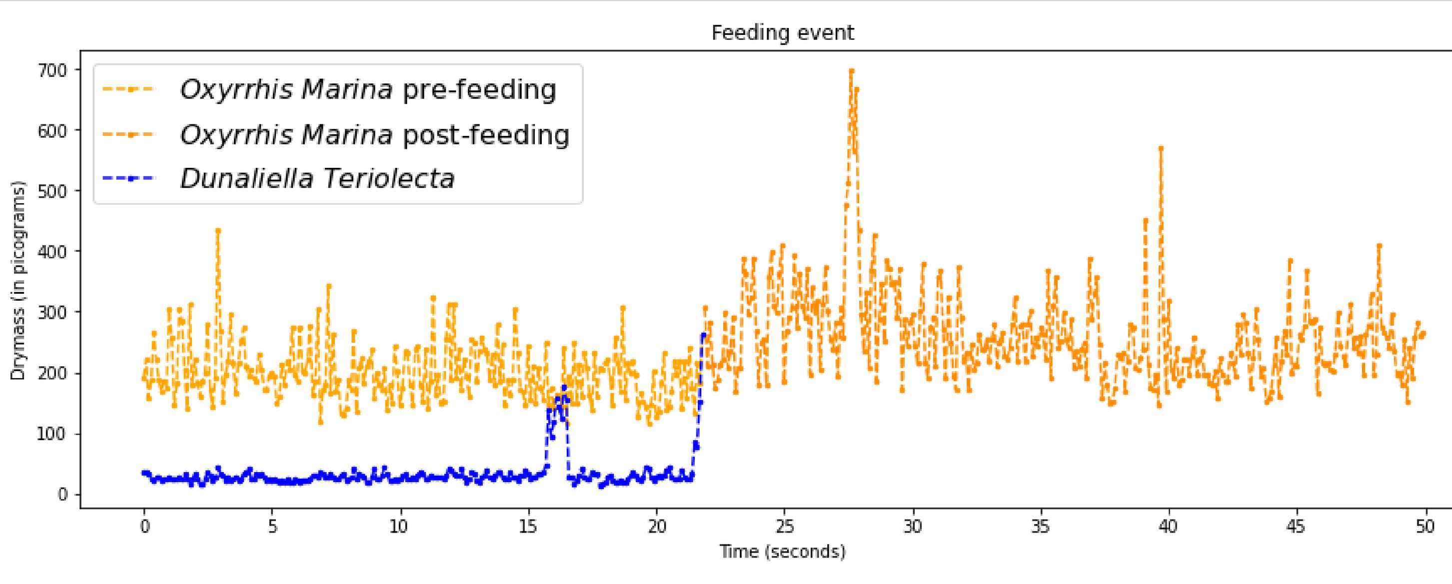
In [9]:
```
prediction_predator = feeding_model.predict(
    Normalise(
        predator_sequence,
        batch = 1
    )
)

prediction_prey = feeding_model.predict(
    Normalise(
        prey_sequence,
        batch = 1
    )
)
```

4.1.3. Convert values to real dry mass units

In [10]:
```
drymass_predator = real_dm(prediction_predator[:,0])
drymass_prey = real_dm(prediction_prey[:,0])
```

4.4. Visualise the dry mass transition in a feeding event

In [11]:
```
feeding_at = 219

fig, ax = plt.subplots(figsize=(15, 5))
plt.title("Feeding event")
plt.plot(np.arange(0,feeding_at,1), drymass_predator[0:feeding_at], linestyle='dashed',color='orange',
marker='.', label='$\it{Oxyrrhis\ Marina}$ pre-feeding', alpha = 1, markersize = '5')
plt.plot(np.arange(feeding_at,len(drymass_predator), 1),drymass_predator[feeding_at:], linestyle='dashe
d',color='darkorange', marker='.', label='$\it{Oxyrrhis\ Marina}$ post-feeding', alpha = 1, markersize
= '5')
plt.plot(drymass_prey[:feeding_at], linestyle='dashed',color='blue', marker='.', label='$\it{Dunaliella
\ Teriolecta}$', alpha=1, markersize = '5')

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.legend(prop={'size': 16})
plt.show()
```



4.2. Example 2 - Double division event

Division events are recorded in three sequences:

- `cell1_sequence` contains frames of parent cell, Gen 1 daughter cell 1, and Gen 2 daughter cell 1
- `cell2_sequence` contains frames of Gen 1 daughter cell 2 (along with parent cell)
- `cell3_sequence` contains frames of Gen 2 daughter cell 2 (along with parent cell and Gen 1 daugher cell 1)

4.2.1. Normalise and predict

Function to de-normalise the dry mass values

In [12]:
```
def dm_range(rad_range, ri_range):
    m = lambda rad, ri: ((4*np.pi)/3) * ((rad*1e+6)**3) * (ri-1.33)
    return m(rad_range[0], ri_range[0]), m(rad_range[1], ri_range[1])
dm_vals = dm_range([4e-6, 9e-6], [1.35, 1.38])
dm_vals
```

Out[12]:
```
(5.361651462126584, 152.6814029644634)
```

Predictions on cell 1 sequence

In [13]:
```
_dm_cell1=[]
for i in range(len(division_models)):

    pred = division_models[i].predict(
        Normalise(
            cell1_sequence,
            batch = 15
        )
    )[:,0]

    pred = real_dm(pred, dm_vals[1]-dm_vals[0], dm_vals[0])
    _dm_cell1.append(pred)

dm_cell1 = np.mean(_dm_cell1, axis=0)
```

Predictions on cell 2 sequence

In [14]:
```
_dm_cell2=[]
for i in range(len(division_models)):

    pred = division_models[i].predict(
        Normalise(
            cell2_sequence,
        )
    )[:,0]

    pred = real_dm(pred, dm_vals[1]-dm_vals[0], dm_vals[0])
    _dm_cell2.append(pred)

dm_cell2 = np.mean(_dm_cell2, axis=0)
```

Predictions on cell 3 sequence

In [15]:
```
_dm_cell3=[]
for i in range(len(division_models)):

    pred = division_models[i].predict(
        Normalise(
            cell3_sequence,
        )
    )[:,0]

    pred = real_dm(pred, dm_vals[1]-dm_vals[0], dm_vals[0])
    _dm_cell3.append(pred)

dm_cell3 = np.mean(_dm_cell3, axis=0)
```

4.2.2. Visualise the dry mass dynamics in a double division event

In [16]:
```
time = np.arange(0, len(dm_cell1), 1)

fig,ax = plt.subplots(figsize=(40, 14))


dt1 = 128
dt2 = 335
end1 = 600

daughter_lim = 10
plt.plot(time[10:dt1], dm_cell1[10:dt1], linestyle='dashed', color="midnightblue",
         marker='.', label='Parent Cell', alpha=1, markersize=10, linewidth=3)  # parentcell1

plt.plot(time[dt1:dt2], dm_cell1[dt1:dt2], linestyle='dashed', color="orange",
         marker='.', label='\nGeneration 1 - Daughter Cell 1', alpha=1, markersize='10', linewidth=3) #
stationary cell

plt.plot(time[dt1:dt2-daughter_lim], dm_cell2[dt1:dt2-daughter_lim], linestyle='dashed', color="orange"
,
         marker='.', label='Generation 1 - Daughter Cell 2', alpha=1, markersize='10', linewidth=3)  #d
isplaced cell


# Second generation----

plt.plot(time[dt2:end1], dm_cell1[dt2:end1], linestyle='dashed', color="darkcyan",
         marker='.', label='\nGeneration 2 - Daughter cell 1', alpha=1, markersize='10', linewidth=3)
# stationary cell

plt.plot(time[dt2:end1], dm_cell3[dt2:end1], linestyle='dashed', color="darkcyan",
         marker='.', label='Generation 2 - Daughter cell 2', alpha=1, markersize='10', linewidth=3)  #
 daughtercell1


def place_ticks(x = 10, y = 10):
    plt.locator_params(axis="x", nbins=x)
    plt.locator_params(axis="y", nbins=y)

xticks = np.arange(0,700,14.5)
ax.set_xticks(xticks)
ax.set_xticklabels([np.round(x*(5/6/60),1) for x in xticks])
place_ticks(x=10, y=10)

fs = 30
plt.xticks(fontsize=fs)
plt.yticks(fontsize=fs)
plt.legend(prop={'size': fs})
plt.ylabel("Dry mass (pg)", fontsize=fs)
plt.xlabel("Time (hours)", fontsize=fs)

plt.ylim([0, 700])
plt.show()
```