

# Deep RNAs applied to Rats tibia



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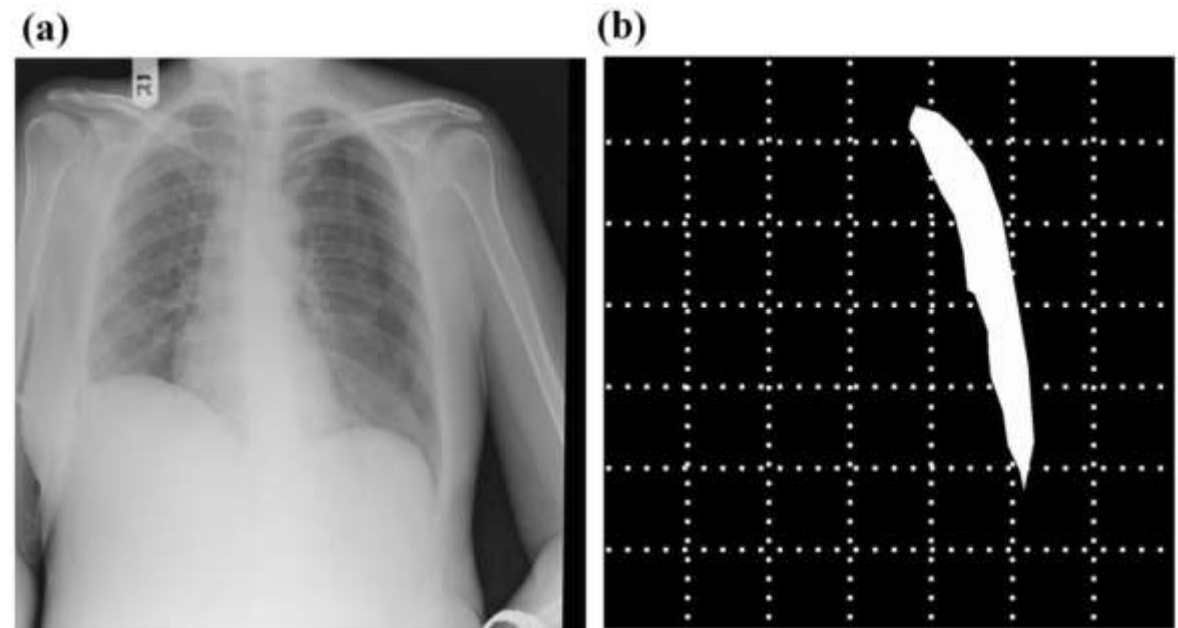
1. Introduction;
  - I. Rats tibia segmentation;
2. Theoretical Background;
  - I. Data acquisition: SR-uCT;
  - II. U-net: Convolutional Networks;
3. Methodology;
  - I. Manual segmentation;
  - II. Choosing Architecture;
  - III. Hyperparameters;
  - IV. Data augmentation and Cross-validation;
4. Conclusion;
  - I. Results and discussion;
  - II. Future objectives.





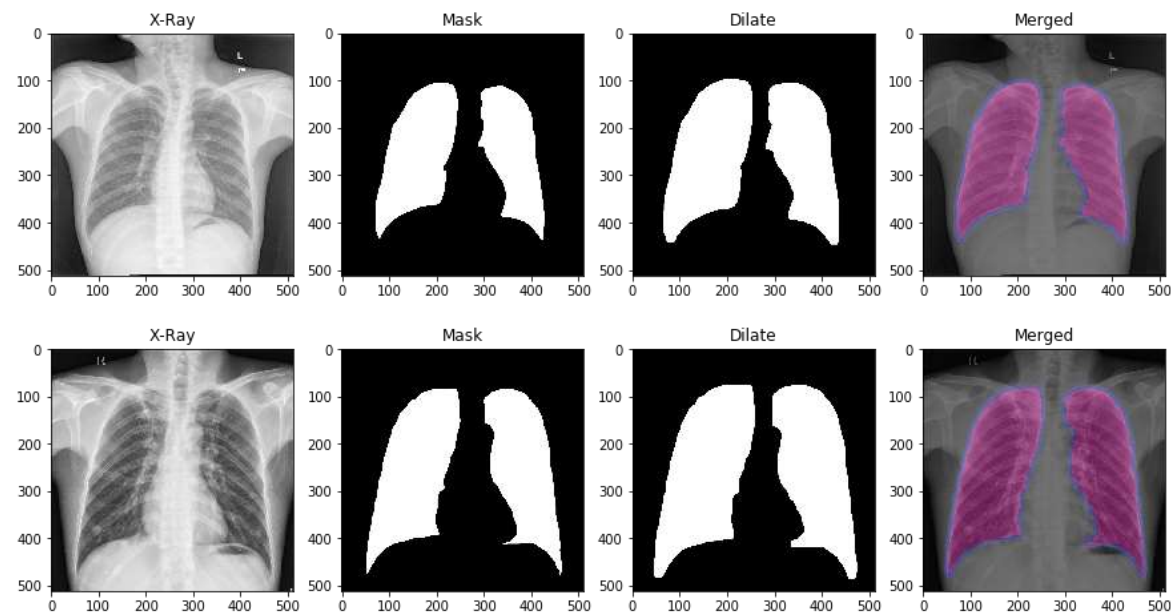
## Biomedical images

Several researches using image segmentation have been carried out in several areas. Among them, we can mention the medical area, where segmentations can be applied to delimit in the image regions such as tumors, cells, glands, organs, cellular tissues, among others. Several segmentation methods can be developed specifically to delimit each of these elements.

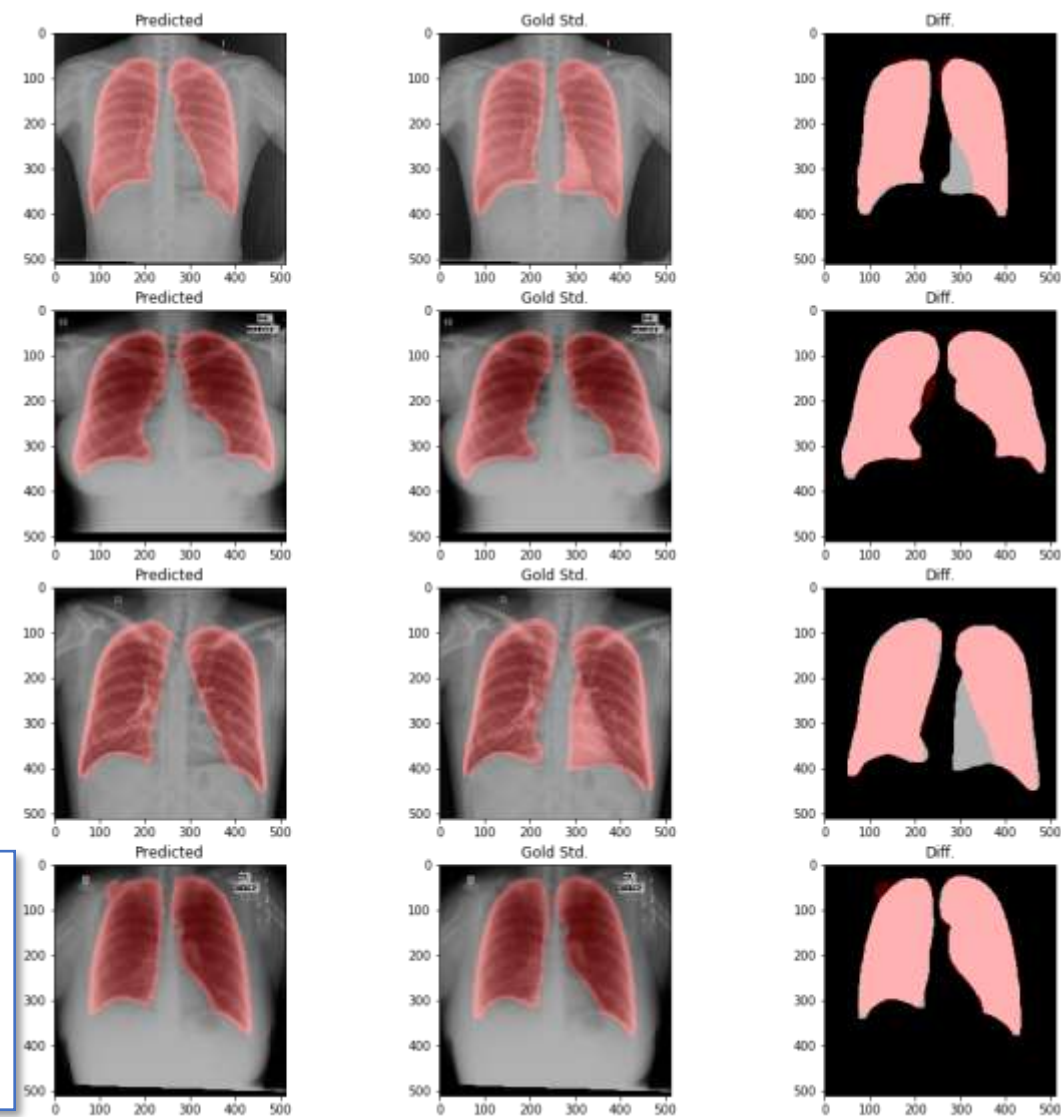


Example of chest X-ray image processing for deep-learning methods. **(a)** Original chest X-ray image. **(b)** Image obtained after marking the location of pneumothorax in white and the remaining areas in black (CHO, 2021) .

## Biomedical images

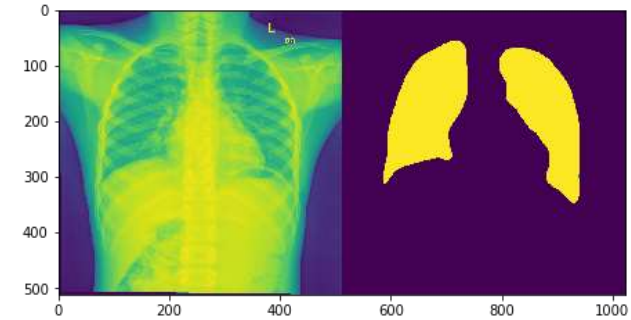
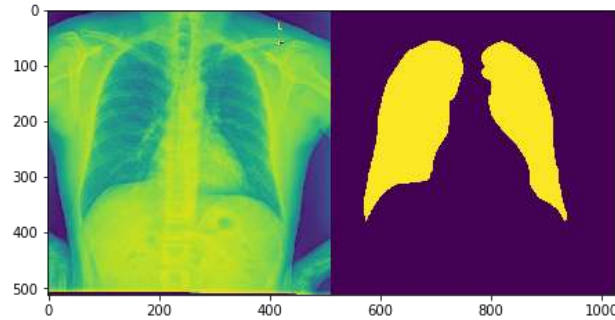
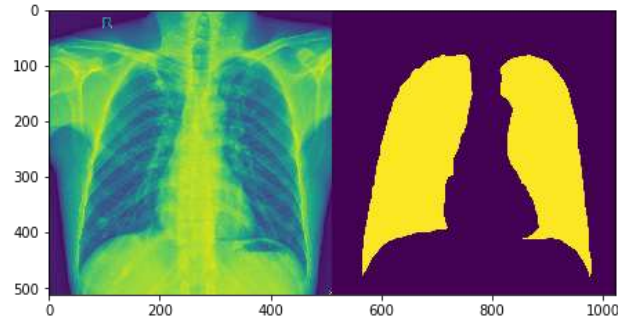
**U-Net lung segmentation (Montgomery + Shenzhen)**

<https://www.kaggle.com/eduardomineo/u-net-lung-segmentation-montgomery-shenzhen/notebook>

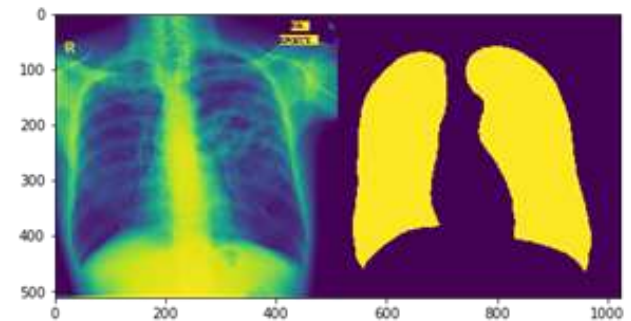
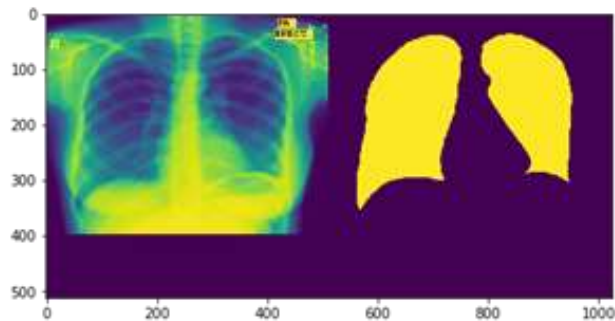
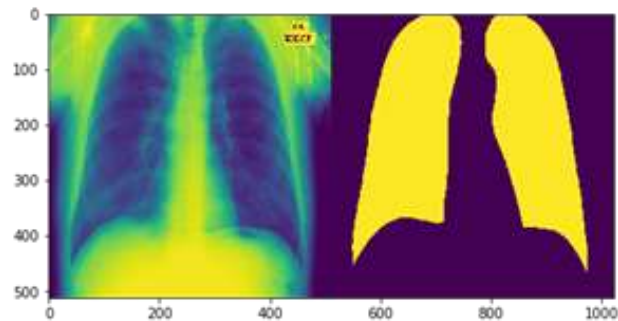


## Biomedical images

Training set



Testing set

**Lung segmentation from Chest X-Ray dataset**

<https://www.kaggle.com/nikhilpandey360/lung-segmentation-from-chest-x-ray-dataset/notebook>



## Biomedical images

## Segmentation of bones in medical dual-energy computed tomography volumes using the 3D U-Net

José Carlos González Sánchez<sup>a</sup>, Maria Magnusson<sup>a,b,c</sup>, Michael Sandborg<sup>a,b</sup>, Åsa Carlsson Tedgren<sup>a,b,d</sup>, Alexandr Malusek<sup>a,b,\*</sup>

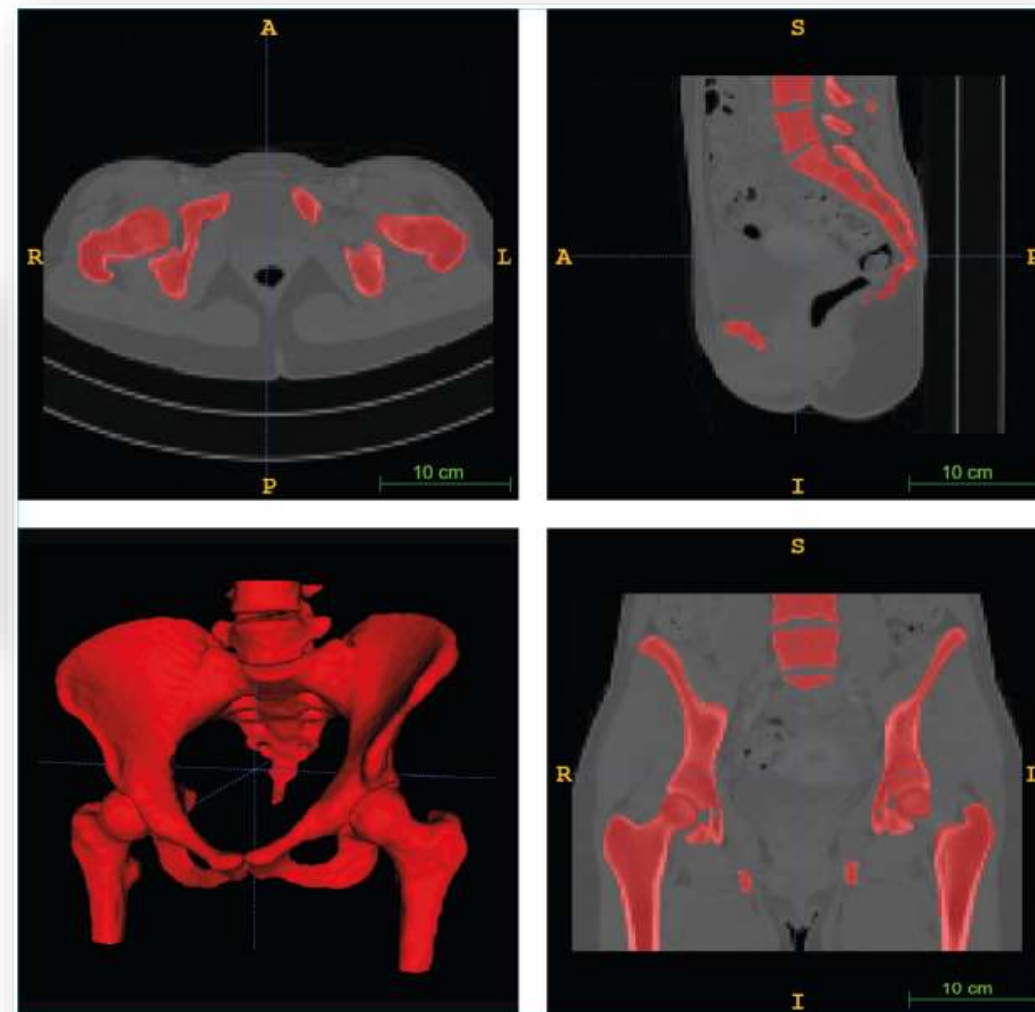
<sup>a</sup> Radiation Physics, Department of Medical and Health Sciences, Linköping University, Linköping SE-581 83, Sweden

<sup>b</sup> Center for Medical Image Science and Visualization (CMIV), Linköping University, Linköping SE-581 83, Sweden

<sup>c</sup> Computer Vision Laboratory, Department of Electrical Engineering, Linköping University, Linköping SE-581 85, Sweden

<sup>d</sup> Department of Medical Radiation Physics and Nuclear Medicine, Karolinska University Hospital, Stockholm SE-171 76, Sweden

(SÁNCHEZ, 2020)



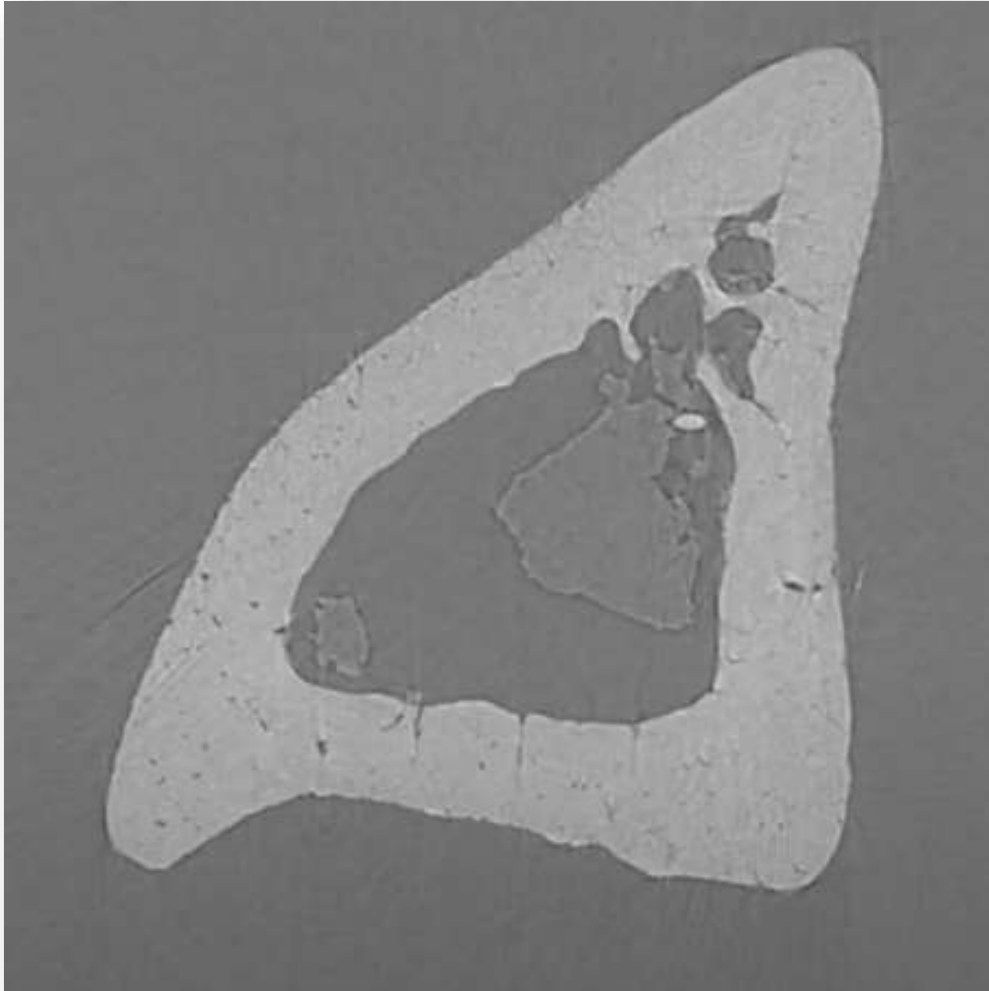
## Rats tibia segmentation

Manual segmentation methods can be very tedious, time-consuming and subject to inter and intraindividual variability ([TINGELHOF, 2008](#)).





## Rats tibia segmentation

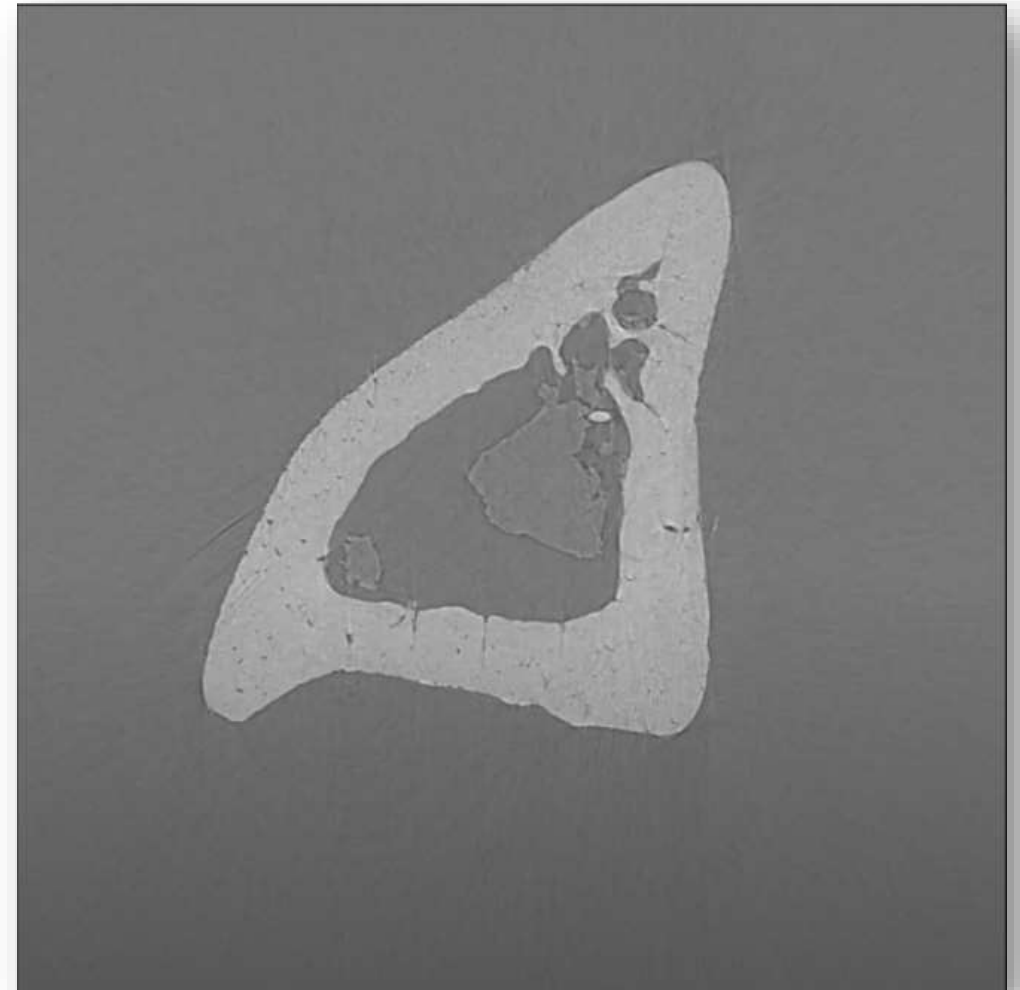


## Rats tibia segmentation

Manual segmentation methods can be very tedious, time-consuming and subject to inter and intraindividual variability ([TINGELHOF, 2008](#)).

Automatic segmentation methods can overcome these problems, nevertheless their performance is in many cases adversely affected by low tissue contrast and image artifacts. Traditional segmentation methods are often based on conventional computer vision and machine learning approaches([GONZALEZ & WOODS, 2018](#))([SÁNCHEZ, 2020](#)).

Recently, methods based on deep learning have demonstrated a potential to notably outperform the traditional methods in medical image analysis ([SÁNCHEZ, 2020](#)).



## Rats tibia segmentation

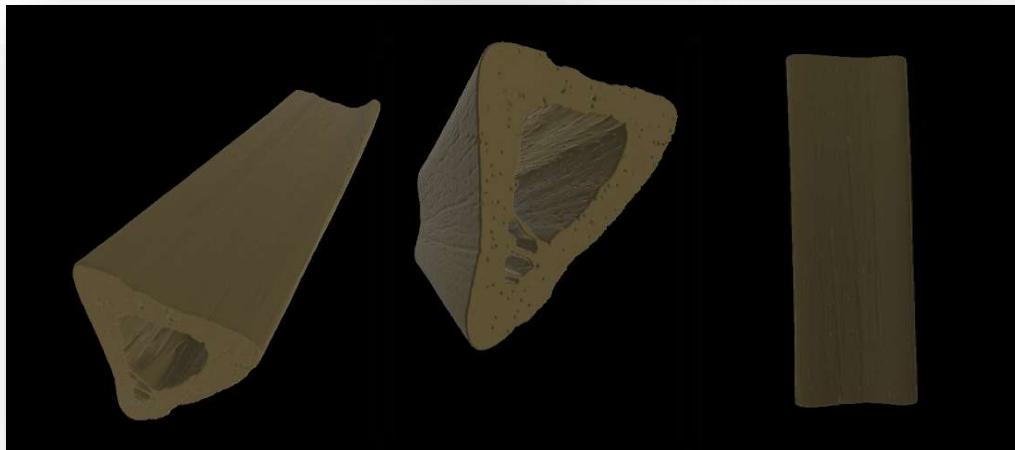
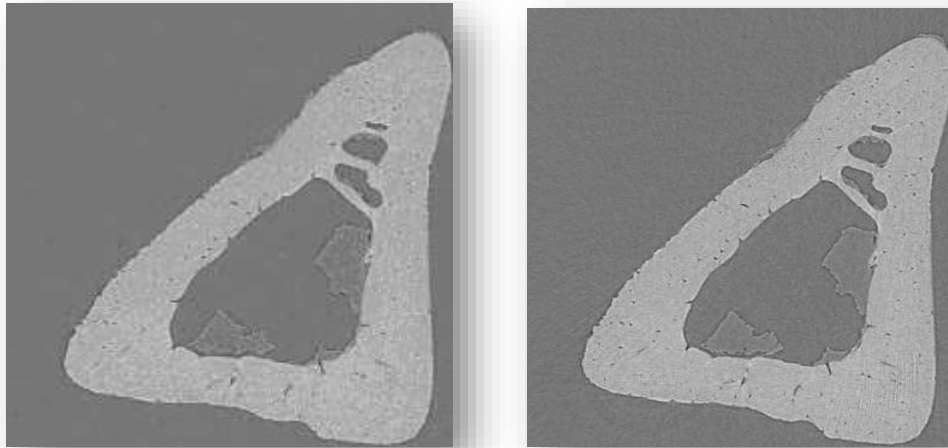
is it possible to segment the rat tibia image by neural networks?



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Theoretical Background

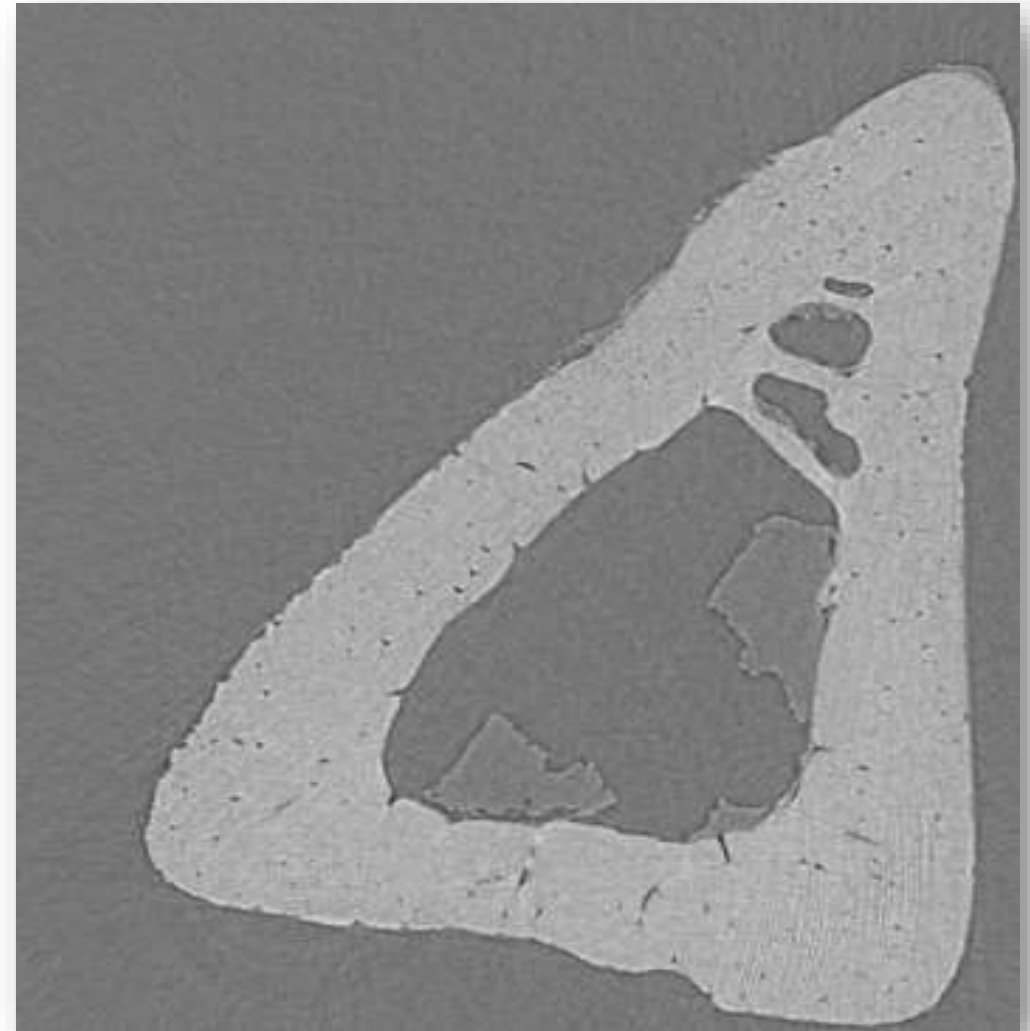
## Data acquisition: SR-uCT



The reconstructed tibial slices. Above, the slices and below they are used to create the 3D volume

- Synchrotron radiation is produced by ring accelerators in which a pulsed stream of high-energy electrons circulates at nearly the speed of light;
- The main characteristic of these synchrotron radiation sources is the large and continuous energy spectrum that provides a high photon flux over an energy range of up to 50 keV or greater;
- The beam has a high natural collimation and a high degree of coherence in space and time. A non-destructive technique that allows visualization of the internal structure of objects;
- According to [Abrami \(2005\)](#), these characteristics in combination with the sophisticated optics differentiate these sources from standard clinical and research tools.

Data acquisition: SR-uCT





## Data acquisition: SR-uCT

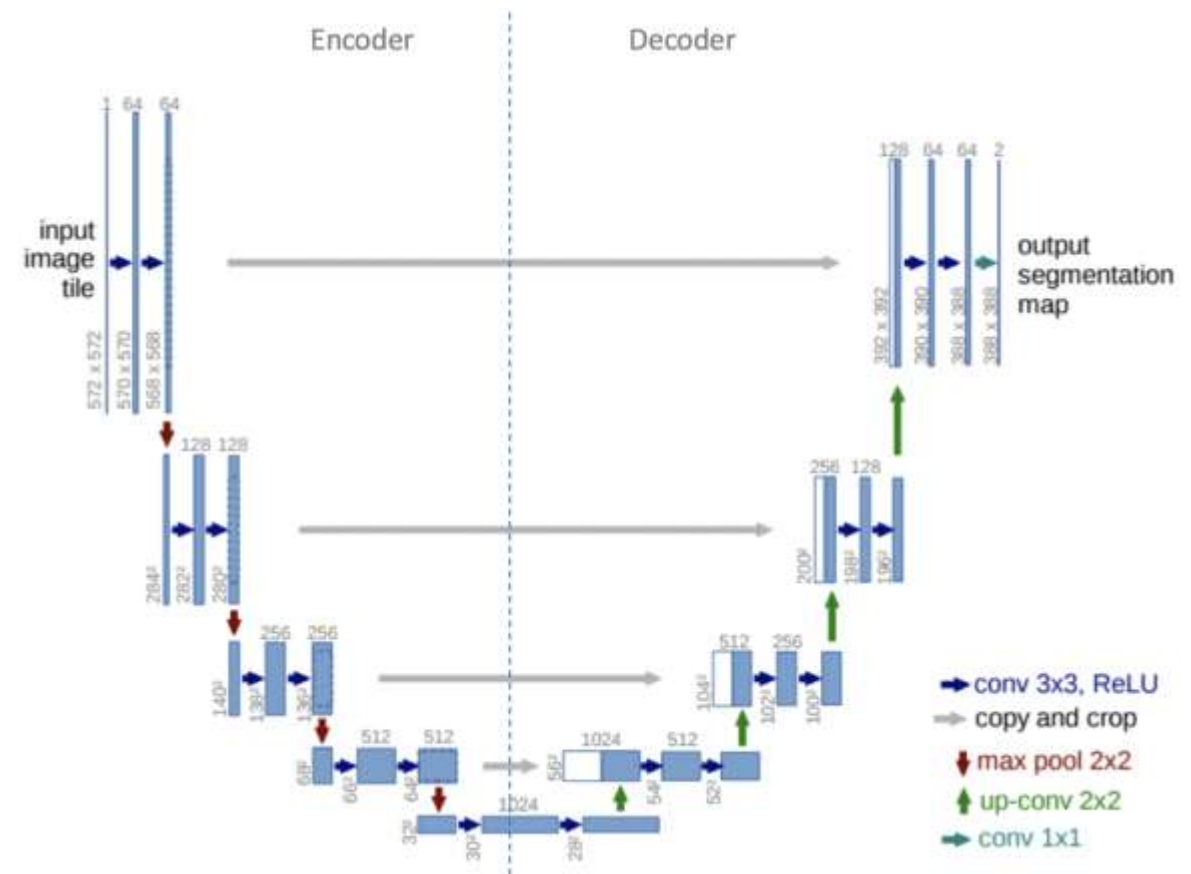


Elettra Sincrotrone Trieste

- According to [Meneses \(2018\)](#), these SR sources provide higher intensity and spatial coherence and therefore higher spatial resolution. It is through these facilities allowed for higher quality images compared to conventional x-ray imaging;
- As per to [Lewis \(2004\)](#) and [Attwood \(2007\)](#), the technique provides better image enhancement and detail and often at lower doses when compared to conventional X-ray techniques.

# U-net: Convolutional Networks

Better segmentation results were achieved when the encoder and decoder parts were organized in levels and connected with skip connections. This design known as the U-Net was first implemented by [Ronneberger et al \(2015\)](#), in 2D and later extended to 3D by [Çiçek \(2016\)](#).

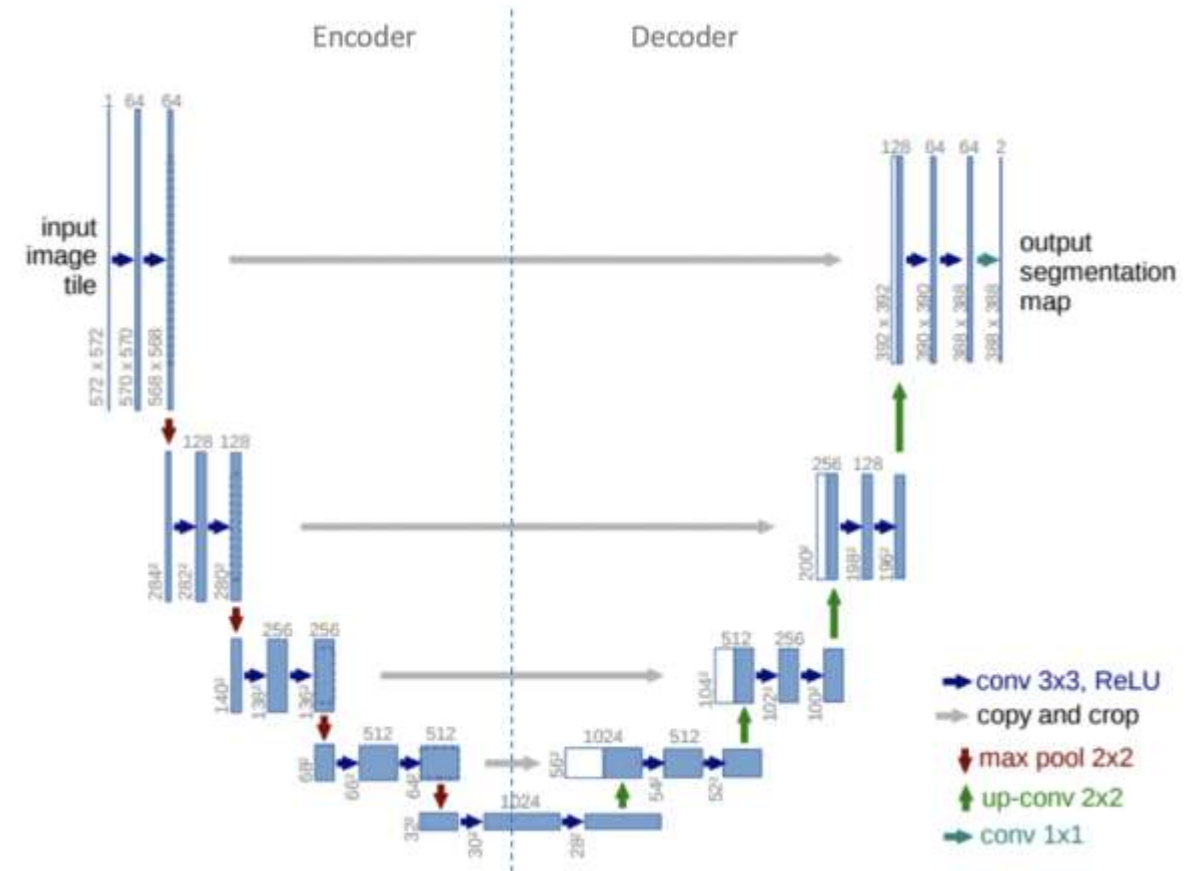


U-net architecture. Blue boxes represent multi-channel feature maps, while white boxes represent copied feature maps. The arrows of different colors represent different operations

# U-net: Convolutional Networks

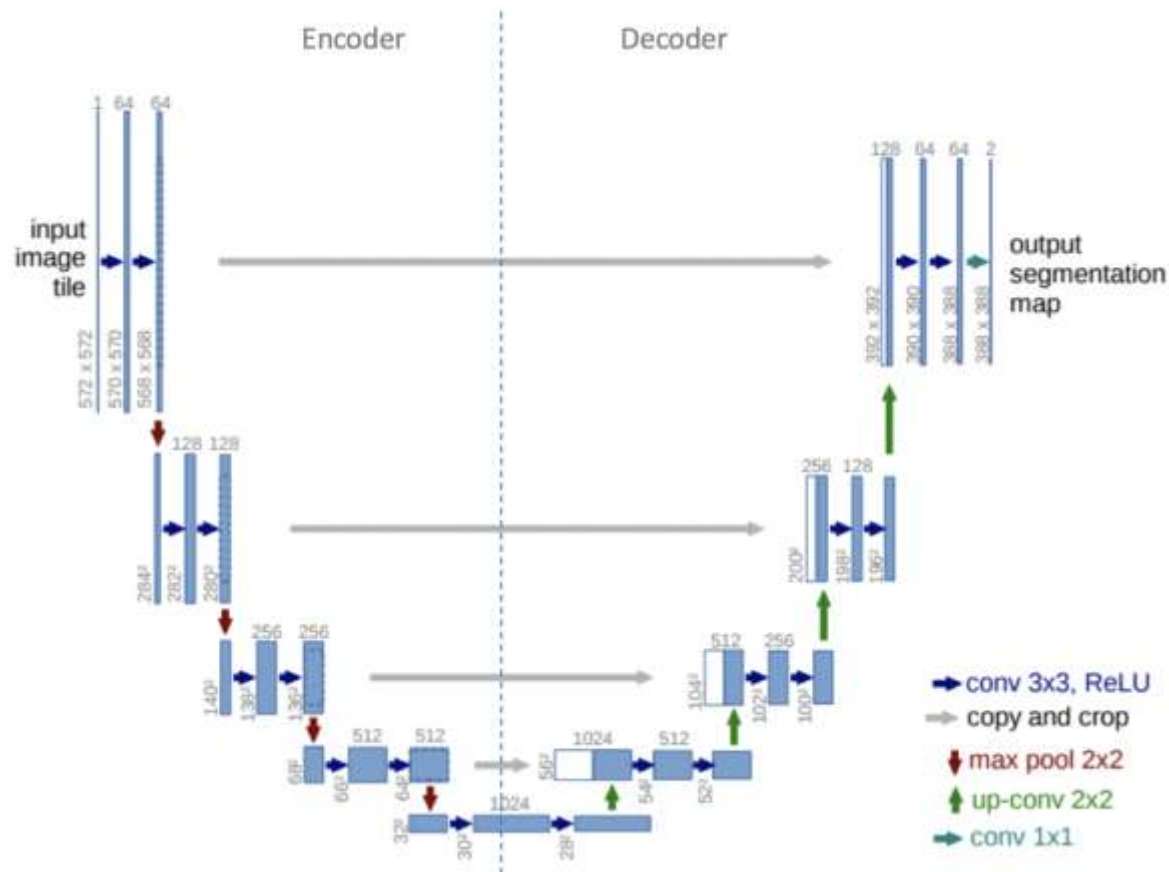
UNet, evolved from the traditional Convolutional Neural Network (CNN), was first designed and applied in 2015 to process biomedical images (ZHANG, 2022).

As a general convolutional neural network focuses its task on image classification, where input is an image and output is one label, but in biomedical cases, it requires us not only to distinguish whether there is a disease, but also to localise the area of abnormality.



U-net architecture. Blue boxes represent multi-channel feature maps, while white boxes represent copied feature maps. The arrows of different colors represent different operations

## U-net: Convolutional Networks



U-net architecture. Blue boxes represent multi-channel feature maps, while white boxes represent copied feature maps. The arrows of different colors represent different operations

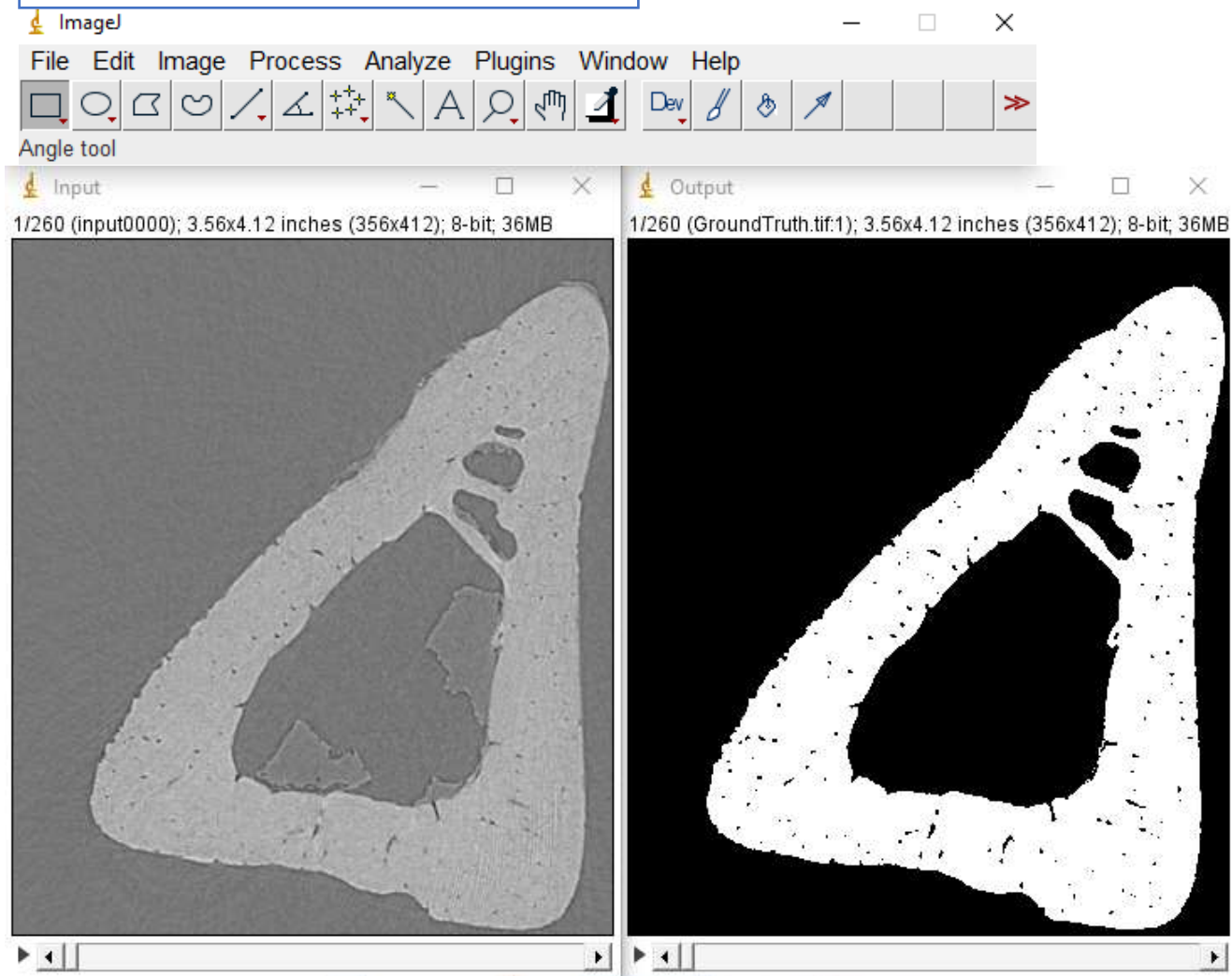
- The network architecture was developed for biomedical images, and consequently has a higher performance for these types of data (RONNEBERGER ET AL, 2015), (MONTE, 2021), (ÇIÇEK, 2016);
- Due to the limited availability of medical images, this neural network was created with the purpose of obtaining efficient results using a small amount of data (MONTE, 2021);
- A significant amount of recent work are using this architecture. (SÁNCHEZ, 2020), (MAIER, 2019) (BREININGE, 2018), (CHEN, 2018) as representative examples;
- Many examples using U-net and easy availability of the code either through Kaggle or Github.

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Methodology

## Manual segmentation

## Training and validation sets



Rat\_24.tif – 260 slices

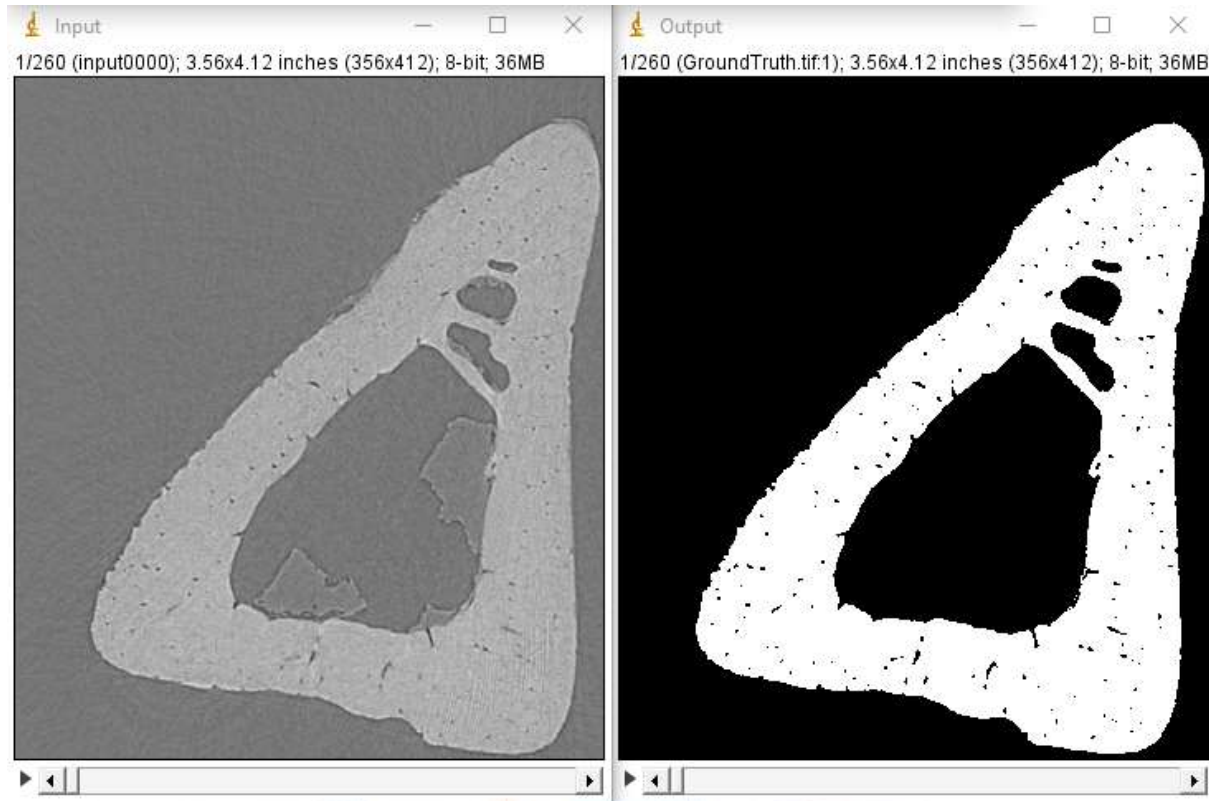
**ImageJ**  
Image Processing and Analysis in Java





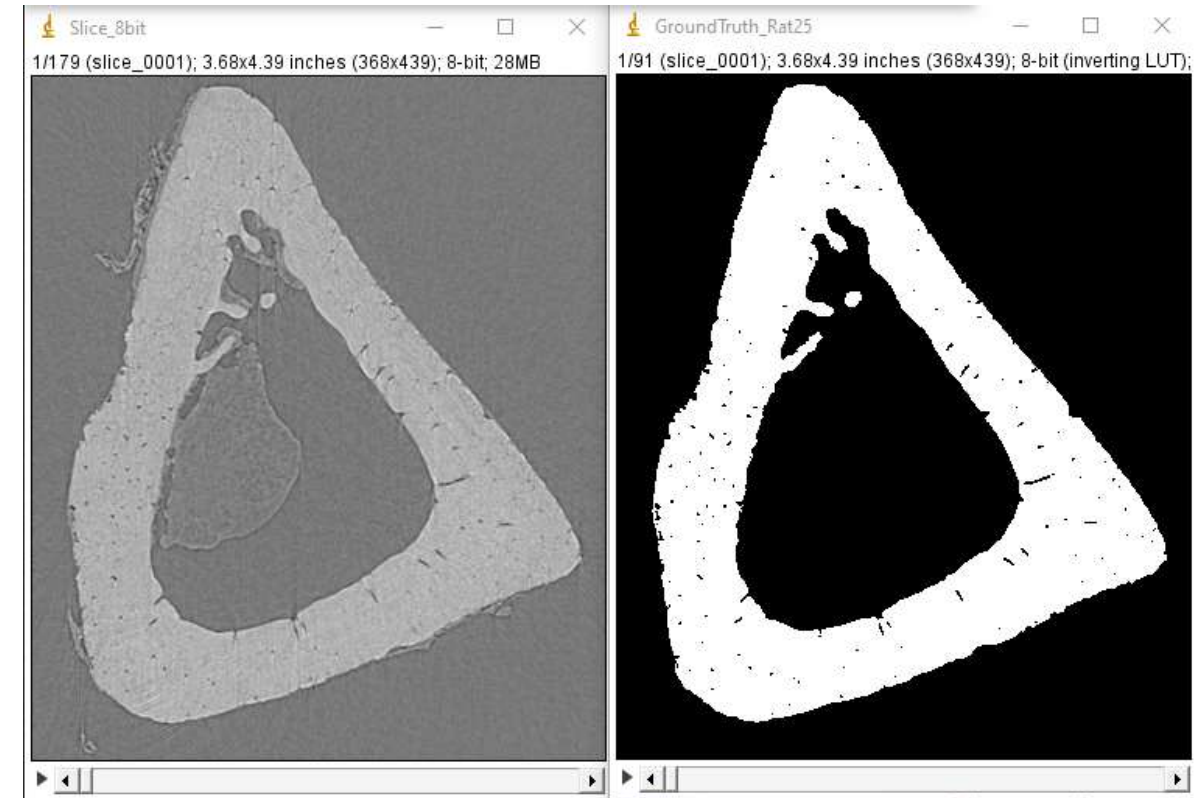
## Manual segmentation

## Training and validation sets



Rat\_24.tif – 260 slices

## Test set



Rat\_25.tif – 179 slices



## Choosing Architecture

## Unet\_Model\_V2 – 12 layers

Layer (type)	Output Shape	Param #	Connected to
input_1 (InputLayer)	[(None, 512, 512, 1, 0)]	0	input_1[0][0]
conv2d (Conv2D)	(None, 512, 512, 16, 160)	160	input_1[0][0]
conv2d_1 (Conv2D)	(None, 512, 512, 16, 2320)	2320	conv2d[0][0]
max_pooling2d (MaxPooling2D)	(None, 256, 256, 16, 0)	0	conv2d_1[0][0]
dropout (Dropout)	(None, 256, 256, 16, 0)	0	max_pooling2d[0][0]
conv2d_2 (Conv2D)	(None, 256, 256, 32, 4640)	4640	dropout[0][0]
conv2d_3 (Conv2D)	(None, 256, 256, 32, 9248)	9248	conv2d_2[0][0]

⋮

⋮

⋮

conv2d_transpose_5 (Conv2DTranspose)	(None, 512, 512, 16, 2320)	2320	conv2d_3[0][0]
concatenate_5 (Concatenate)	(None, 512, 512, 32, 0)	0	conv2d_transpose_5[0][0], conv2d_1[0][0]
dropout_11 (Dropout)	(None, 512, 512, 32, 0)	0	concatenate_5[0][0]
conv2d_24 (Conv2D)	(None, 512, 512, 16, 4624)	4624	dropout_11[0][0]
conv2d_25 (Conv2D)	(None, 512, 512, 16, 2320)	2320	conv2d_24[0][0]
conv2d_26 (Conv2D)	(None, 512, 512, 1, 17)	17	conv2d_25[0][0]

Total params: 34,585,649  
 Trainable params: 34,585,649  
 Non-trainable params: 0

## Unet\_Model\_V1 – 8 layers

Layer (type)	Output Shape	Param #	Connected to
input_1 (InputLayer)	[(None, 512, 512, 1, 0)]	0	input_1[0][0]
conv2d (Conv2D)	(None, 512, 512, 16, 160)	160	input_1[0][0]
conv2d_1 (Conv2D)	(None, 512, 512, 16, 2320)	2320	conv2d[0][0]
max_pooling2d (MaxPooling2D)	(None, 256, 256, 16, 0)	0	conv2d_1[0][0]
dropout (Dropout)	(None, 256, 256, 16, 0)	0	max_pooling2d[0][0]
conv2d_2 (Conv2D)	(None, 256, 256, 32, 4640)	4640	dropout[0][0]
conv2d_3 (Conv2D)	(None, 256, 256, 32, 9248)	9248	conv2d_2[0][0]
max_pooling2d_1 (MaxPooling2D)	(None, 128, 128, 32, 0)	0	conv2d_3[0][0]

⋮

⋮

⋮

conv2d_15 (Conv2D)	(None, 256, 256, 32, 9248)	9248	conv2d_14[0][0]
conv2d_transpose_3 (Conv2DTranspose)	(None, 512, 512, 16, 4624)	4624	conv2d_15[0][0]
concatenate_3 (Concatenate)	(None, 512, 512, 32, 0)	0	conv2d_transpose_3[0][0], conv2d_1[0][0]
dropout_7 (Dropout)	(None, 512, 512, 32, 0)	0	concatenate_3[0][0]
conv2d_16 (Conv2D)	(None, 512, 512, 16, 4624)	4624	dropout_7[0][0]
conv2d_17 (Conv2D)	(None, 512, 512, 16, 2320)	2320	conv2d_16[0][0]
conv2d_18 (Conv2D)	(None, 512, 512, 1, 17)	17	conv2d_17[0][0]

Total params: 2,158,417  
 Trainable params: 2,158,417  
 Non-trainable params: 0

## Choosing Architecture

## Unet\_Model\_V2 – 12 layers

Layer (type)	Output Shape	Param #	Connected to
input_1 (InputLayer)	[(None, 512, 512, 1, 0)]		[]
conv2d (Conv2D)	(None, 512, 512, 16, 160)		['input_1[0][0]']
conv2d_1 (Conv2D)	(None, 512, 512, 16, 2320)		['conv2d[0][0]']
max_pooling2d (MaxPooling2D)	(None, 256, 256, 16, 0)		['conv2d_1[0][0]']
dropout (Dropout)	(None, 256, 256, 16, 0)		['max_pooling2d[0][0]']
conv2d_2 (Conv2D)	(None, 256, 256, 32, 4640)		['dropout[0][0]']
conv2d_3 (Conv2D)	(None, 256, 256, 32, 9248)		['conv2d_2[0][0]']

⋮

⋮

⋮

conv2d_transpose_5 (Conv2DTranspose)	(None, 512, 512, 16, 2320)		['conv2d_3[0][0]']
concatenate_5 (Concatenate)	(None, 512, 512, 32, 0)		['conv2d_transpose_5[0][0]', 'conv2d_1[0][0]']
dropout_11 (Dropout)	(None, 512, 512, 32, 0)		['concatenate_5[0][0]']
conv2d_24 (Conv2D)	(None, 512, 512, 16, 4624)		['dropout_11[0][0]']
conv2d_25 (Conv2D)	(None, 512, 512, 16, 2320)		['conv2d_24[0][0]']
conv2d_26 (Conv2D)	(None, 512, 512, 1, 17)		['conv2d_25[0][0]']
Total params: 34,585,649			
Trainable params: 34,585,649			
Non-trainable params: 0			

```
# 1st layer: convolution
# 512 -> 256
conv1_1 = Conv2D(input_filter, input_kernel_size, activation='relu', padding="same")(input_layer)
conv1_1 = Conv2D(input_filter, input_kernel_size, activation='relu', padding="same")(conv1_1)
if pool_type == 'max':
    pool1_1 = MaxPooling2D(pool_size=(2, 2))(conv1_1)
if pool_type == 'average':
    pool1_1 = AveragePooling2D(pool_size=(2, 2))(conv1_1)
pool1_1 = Dropout(rate=dropout_rate)(pool1_1)
```

```
# 2nd layer: convolution
# 256 -> 128
conv1 = Conv2D(input_filter*2, third_kernel_size, padding="same", activation=activation)(pool1_1)
conv1 = Conv2D(input_filter*2, third_kernel_size, padding="same", activation=activation)(conv1)
if pool_type == 'max':
    pool1 = MaxPooling2D(pool_size=(2, 2))(conv1)
if pool_type == 'average':
    pool1 = AveragePooling2D(pool_size=(2, 2))(conv1)
pool1 = Dropout(rate=0.5)(pool1)
```

⋮

⋮

⋮

```
# 6th layer: convolution
# 16 -> 8
conv5 = Conv2D(input_filter*32, third_kernel_size, padding="same", activation=activation)(pool4)
conv5 = Conv2D(input_filter*32, third_kernel_size, padding="same", activation=activation)(conv5)
if pool_type == 'max':
    pool5 = MaxPooling2D(pool_size=(2, 2))(conv5)
if pool_type == 'average':
    pool5 = AveragePooling2D(pool_size=(2, 2))(conv5)
pool5 = Dropout(rate=0.5)(pool5)
```



## Choosing Architecture

```
# 1st layer: convolution
# 512 -> 256
conv1_1 = Conv2D(input_filter, input_kernel_size, activation='relu', padding="same")(input_layer)
conv1_1 = Conv2D(input_filter, input_kernel_size, activation='relu', padding="same")(conv1_1)
if pool_type == 'max':
    pool1_1 = MaxPooling2D(pool_size=(2, 2))(conv1_1)
if pool_type == 'average':
    pool1_1 = AveragePooling2D(pool_size=(2, 2))(conv1_1)
pool1_1 = Dropout(rate=dropout_rate)(pool1_1)
```

```
# 2nd layer: convolution
# 256 -> 128

conv1 = Conv2D(input_filter*2, third_kernel_size, padding="same", activation=activation)(pool1_1)
conv1 = Conv2D(input_filter*2, third_kernel_size, padding="same", activation=activation)(conv1)
if pool_type == 'max':
    pool1 = MaxPooling2D(pool_size=(2, 2))(conv1)
if pool_type == 'average':
    pool1 = AveragePooling2D(pool_size=(2, 2))(conv1)
pool1 = Dropout(rate=0.5)(pool1)
```

```
# 4th layer: convolution
# 64 -> 32

conv3 = Conv2D(input_filter*8, third_kernel_size, padding="same", activation=activation)(pool2)
conv3 = Conv2D(input_filter*8, third_kernel_size, padding="same", activation=activation)(conv3)
if pool_type == 'max':
    pool3 = MaxPooling2D(pool_size=(2, 2))(conv3)
if pool_type == 'average':
    pool3 = AveragePooling2D(pool_size=(2, 2))(conv3)
pool3 = Dropout(rate=0.5)(pool3)
```

## Unet\_Model\_V1 – 8 layers

Layer (type)	Output Shape	Param #	Connected to
input_1 (InputLayer)	(None, 512, 512, 1)	0	input_1[0][0]
conv2d (Conv2D)	(None, 512, 512, 16)	160	conv2d[0][0]
conv2d_1 (Conv2D)	(None, 512, 512, 16)	2320	conv2d_1[0][0]
max_pooling2d (MaxPooling2D)	(None, 256, 256, 16)	0	max_pooling2d[0][0]
dropout (Dropout)	(None, 256, 256, 16)	0	dropout[0][0]
conv2d_2 (Conv2D)	(None, 256, 256, 32)	4640	conv2d_2[0][0]
conv2d_3 (Conv2D)	(None, 256, 256, 32)	9248	conv2d_3[0][0]
max_pooling2d_1 (MaxPooling2D)	(None, 128, 128, 32)	0	max_pooling2d_1[0][0]
⋮	⋮	⋮	⋮
conv2d_15 (Conv2D)	(None, 256, 256, 32)	9248	conv2d_15[0][0]
conv2d_transpose_3 (Conv2DTranspose)	(None, 512, 512, 16)	4624	conv2d_transpose_3[0][0]
concatenate_3 (Concatenate)	(None, 512, 512, 32)	0	concatenate_3[0][0]
dropout_7 (Dropout)	(None, 512, 512, 32)	0	dropout_7[0][0]
conv2d_16 (Conv2D)	(None, 512, 512, 16)	4624	conv2d_16[0][0]
conv2d_17 (Conv2D)	(None, 512, 512, 16)	2320	conv2d_17[0][0]
conv2d_18 (Conv2D)	(None, 512, 512, 1)	17	conv2d_18[0][0]
Total params: 2,158,417 Trainable params: 2,158,417 Non-trainable params: 0			

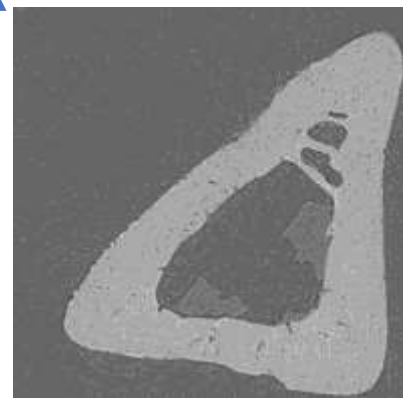
## Hyperparameters

```
def U_net(input_layer,  
         input_filter = 16,  
         input_kernel_size = (3,3),  
         second_kernel_size = (3,3),  
         third_kernel_size = (3,3),  
         pool_type = 'max',  
         activation = 'tanh',  
         optimizer = 'SGD',  
         dropout_rate = 0.25):
```

```
# 1st layer: convolution  
# 512 -> 256  
conv1_1 = Conv2D(input_filter, input_kernel_size, activation='relu', padding="same")(input_layer)  
conv1_1 = Conv2D(input_filter, input_kernel_size, activation='relu', padding="same")(conv1_1)  
if pool_type == 'max':  
    pool1_1 = MaxPooling2D(pool_size=(2, 2))(conv1_1)  
if pool_type == 'average':  
    pool1_1 = AveragePooling2D(pool_size=(2, 2))(conv1_1)  
pool1_1 = Dropout(rate=dropout_rate)(pool1_1)
```

```
#output  
output_layer = Conv2D(1, (1,1), padding="same", activation="sigmoid")(uconv1_1)  
  
return output_layer
```

512 px



512 px

```
# Compile the model  
un.model.compile(optimizer= 'Adam',  
                 loss= un.dice_coef_loss,  
                 metrics = [un.dice_coef])
```

## Hyperparameters

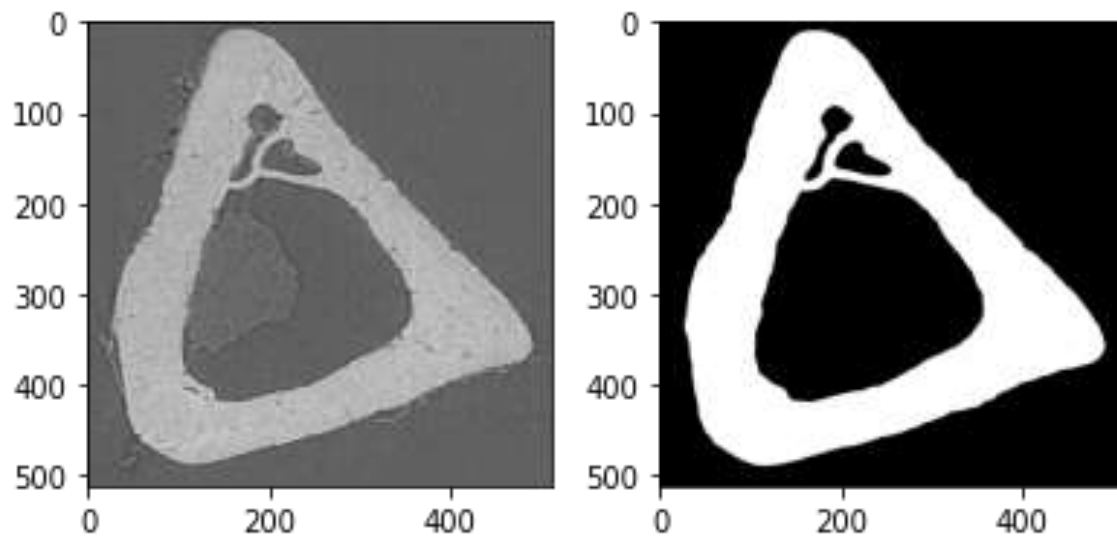
```
# Model configuration  
batch_size = 2  
no_classes = 100  
no_epochs = 100  
verbosity = 0
```

```
# Fit data to model  
loss_history = un.model.fit(inputs[train], targets[train],  
                             batch_size=batch_size,  
                             epochs=no_epochs,  
                             validation_data = [inputs[test], targets[test]],  
                             callbacks=callbacks_list)
```



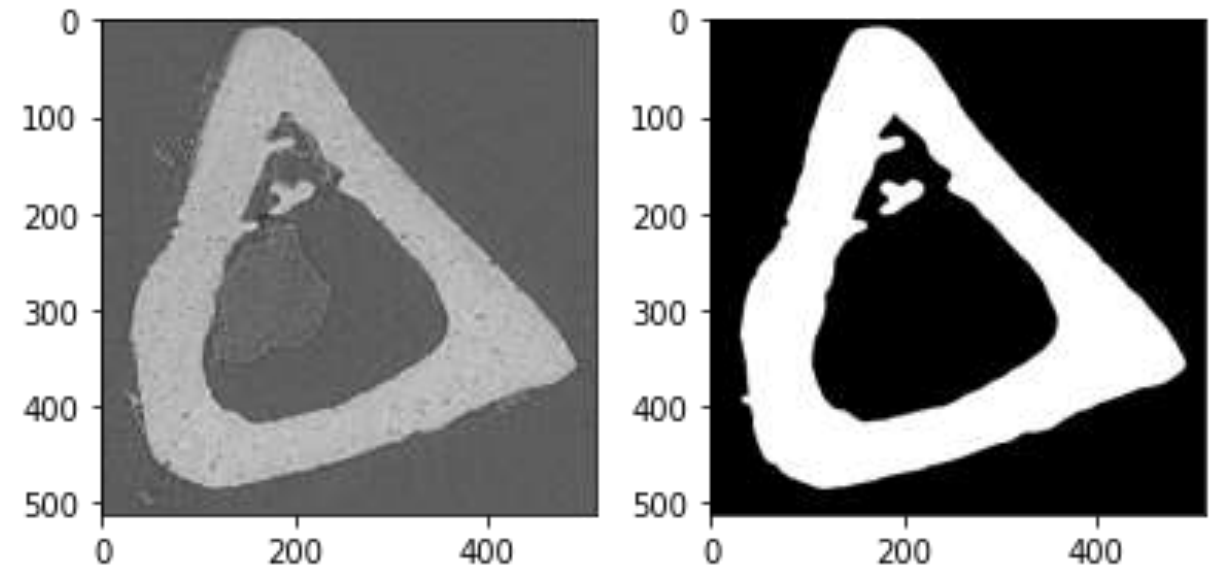
## Hyperparameters

batch\_size = 5, epochs = 100



Dice coefficient : (57 +/-25)%

batch\_size = 1, epochs = 100



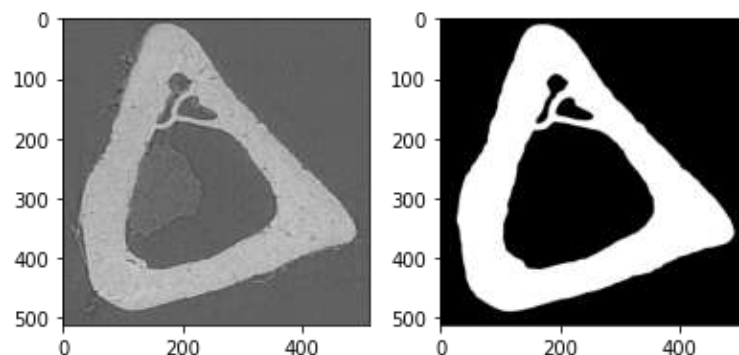
Dice coefficient : (76 +/- 7)%

## Hyperparameters

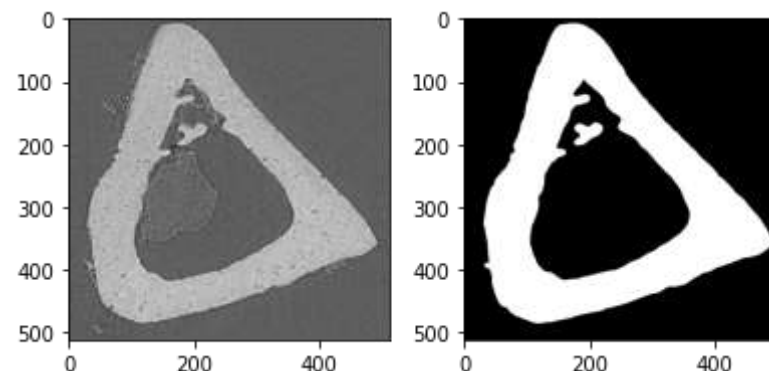
*#Callbacks, Early Stopping and Reduced LR-----*

```
checkpoint = ModelCheckpoint(weight_path, monitor='val_loss', verbose=1,  
                             save_best_only=True, mode='min', save_weights_only = True)  
  
reduceLROnPlat = ReduceLROnPlateau(monitor='val_loss', factor=0.5,  
                                    patience=3,  
                                    verbose=1, mode='min', min_delta=0.0001, cooldown=2, min_lr=1e-6)  
  
early = EarlyStopping(monitor="val_loss",  
                      mode="min",  
                      patience=20,  
                      restore_best_weights=True)  
  
callbacks_list = [checkpoint, early, reduceLROnPlat]
```

batch\_size = 5, epochs = 100



batch\_size = 1, epochs = 100

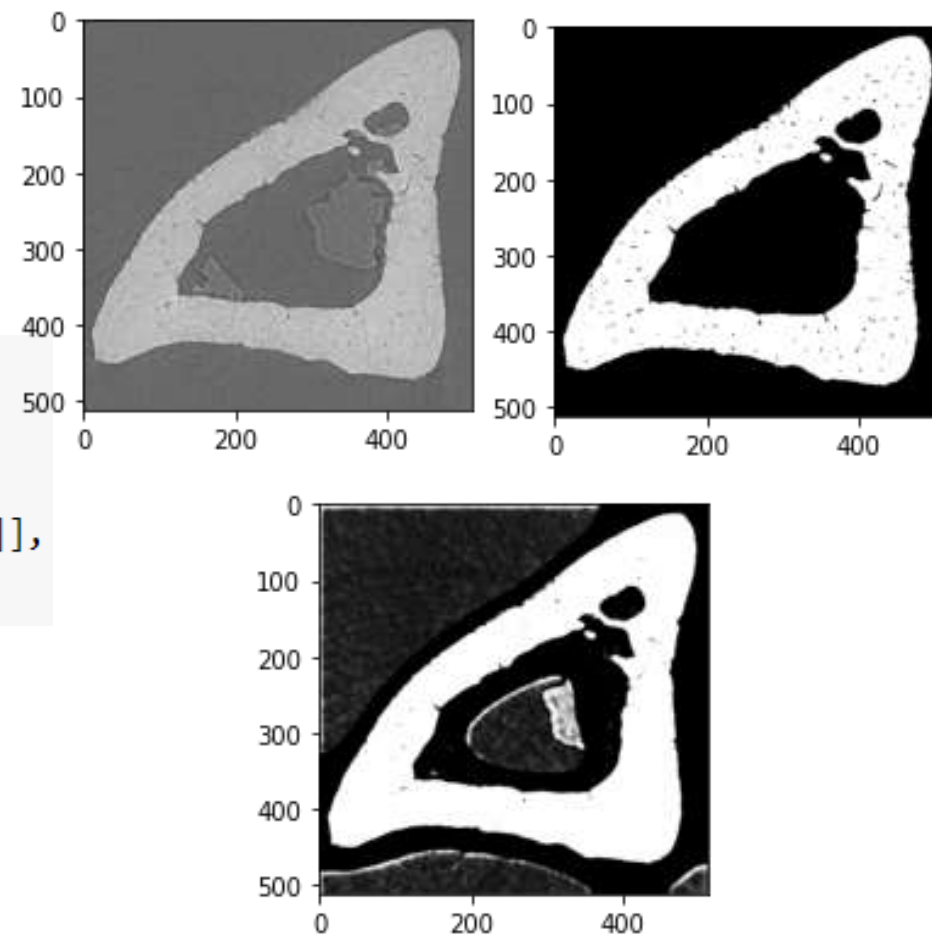


## Hyperparameters

```
# Model configuration
batch_size = 2
no_classes = 100
no_epochs = 100
verbosity = 0
```

```
# Fit data to model
loss_history = un.model.fit(inputs[train], targets[train],
                             batch_size=batch_size,
                             epochs=no_epochs,
                             validation_data = [inputs[test], targets[test]],
                             callbacks=callbacks_list)
```

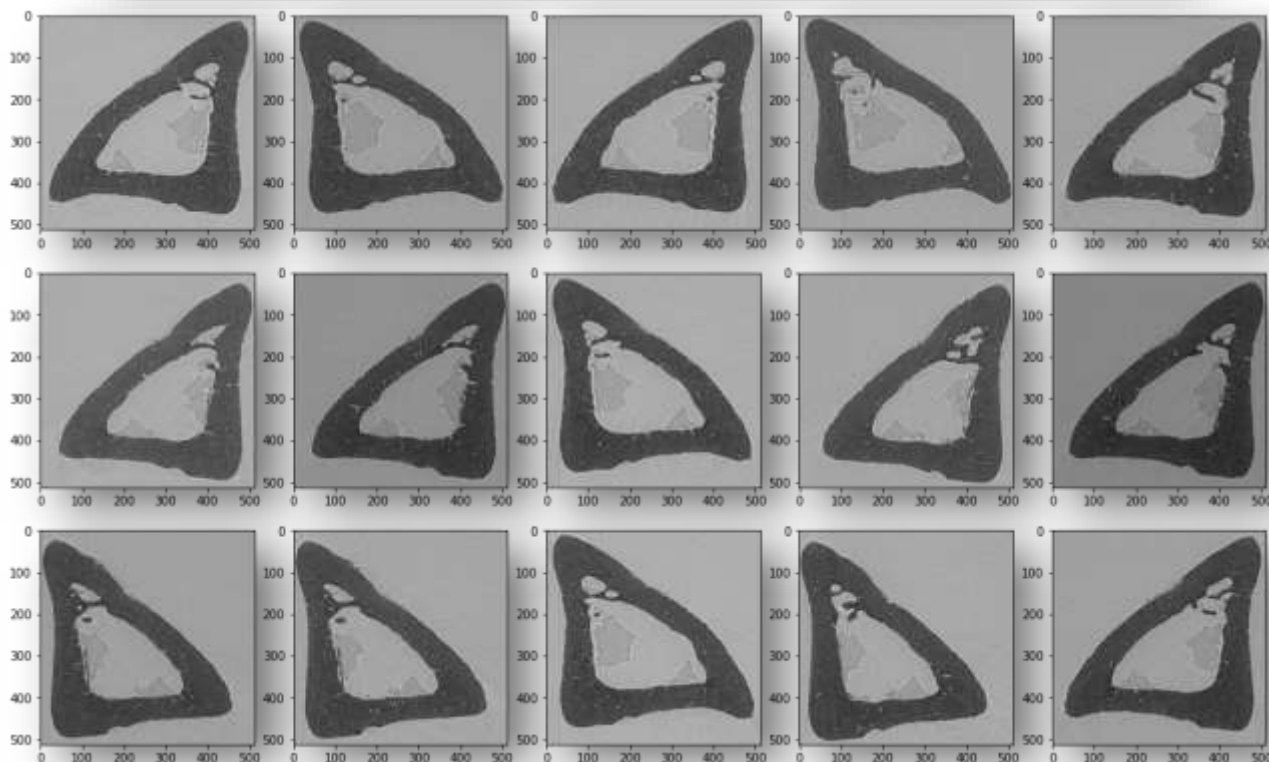
- It has been empirically observed that smaller batch sizes not only have faster training dynamics, but also generalization to the test dataset versus larger batch sizes ([deep learning book, 2022](#)).



batch\_size = 10, epochs = 100

## Data augmentation and Cross-validation

```
import numpy as np
input = np.append(X, [np.fliplr(x) for x in X], axis=0)
output = np.append(y, [np.fliplr(x) for x in y], axis=0)
```



```
# Define the K-fold Cross Validator
```

```
kfold = KFold(n_splits=num_folds,
              shuffle=True,
              random_state = 18)
```

```
# K-fold Cross Validation model evaluation
```

```
fold_no = 10
```

```
for train, test in kfold.split(inputs, targets):
```

```
    :
```

```
    :
```

```
    :
```

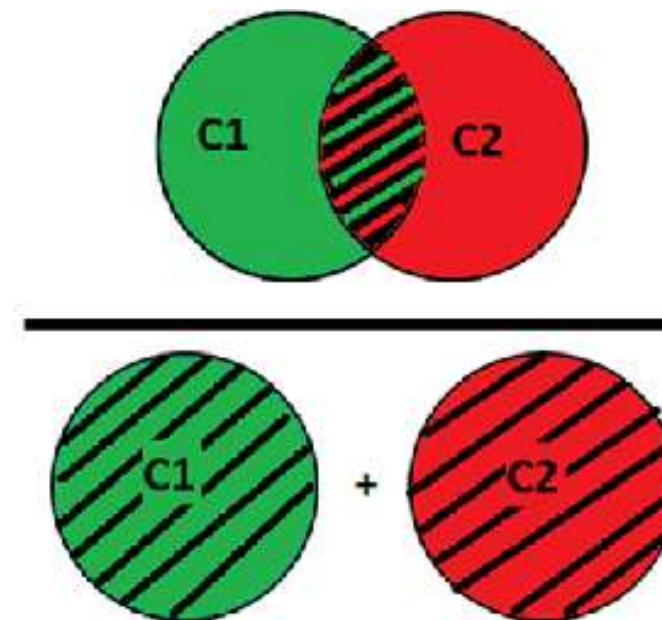
Dice coefficiente

$$D = \frac{2 \sum_i^N p_i g_i}{\sum_i^N p_i^2 + \sum_i^N g_i^2}$$

Dice coefficiente

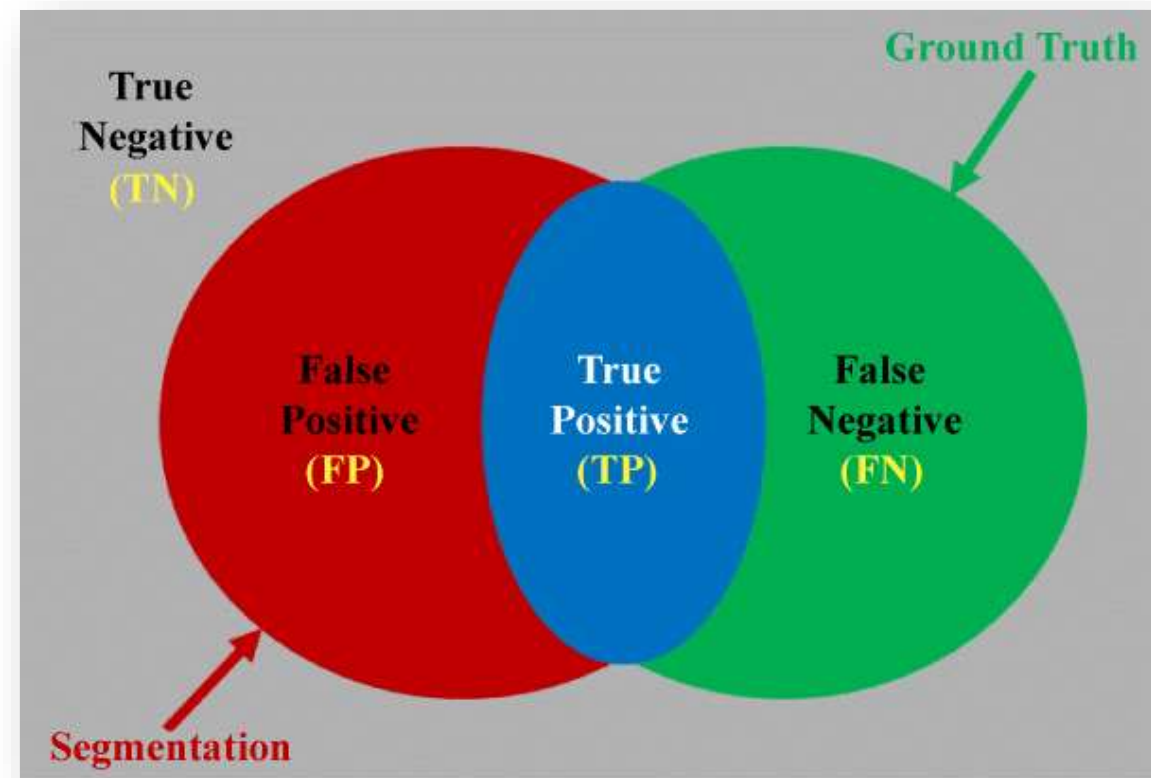
$$D = \frac{2 \sum_i^N p_i g_i}{\sum_i^N p_i^2 + \sum_i^N g_i^2}$$

Dice = 2 x



Dice coefficiente

$$D = \frac{2 \sum_i^N p_i g_i}{\sum_i^N p_i^2 + \sum_i^N g_i^2}$$





DEEP RNAS APPLIED TO RATS TIBIA

Conclusion

is it possible to segment the rat tibia image by neural networks?



## Results and discussion

Rat 25 - GroundTruth

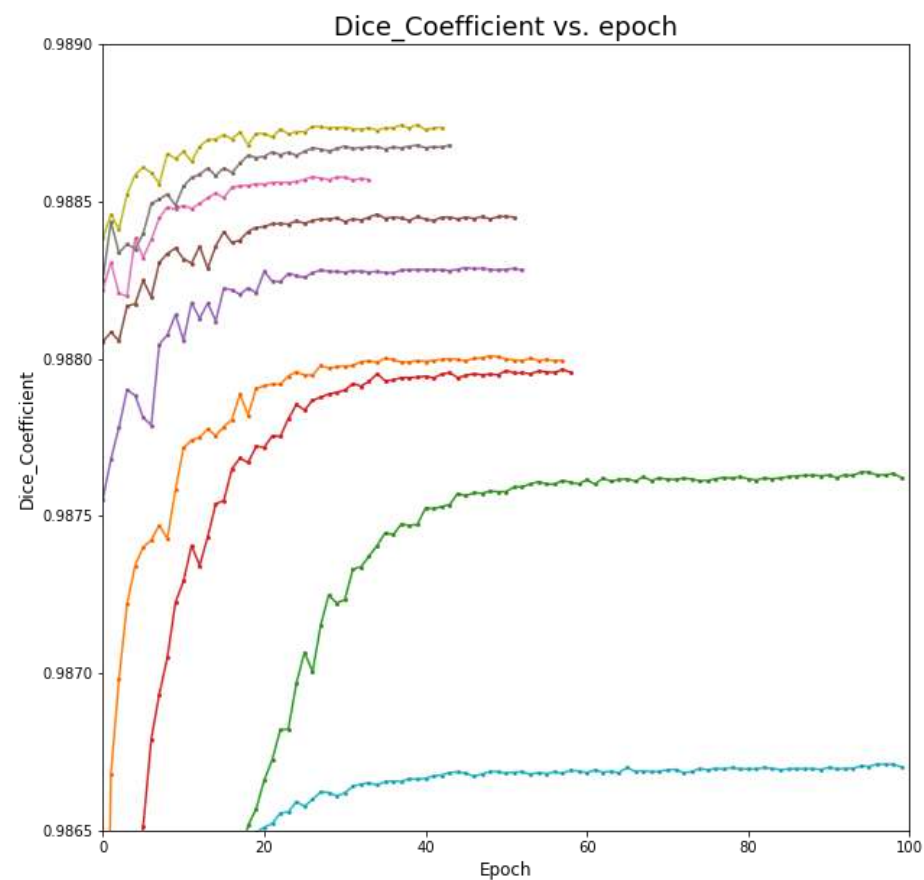


Rat 25 - Predicted

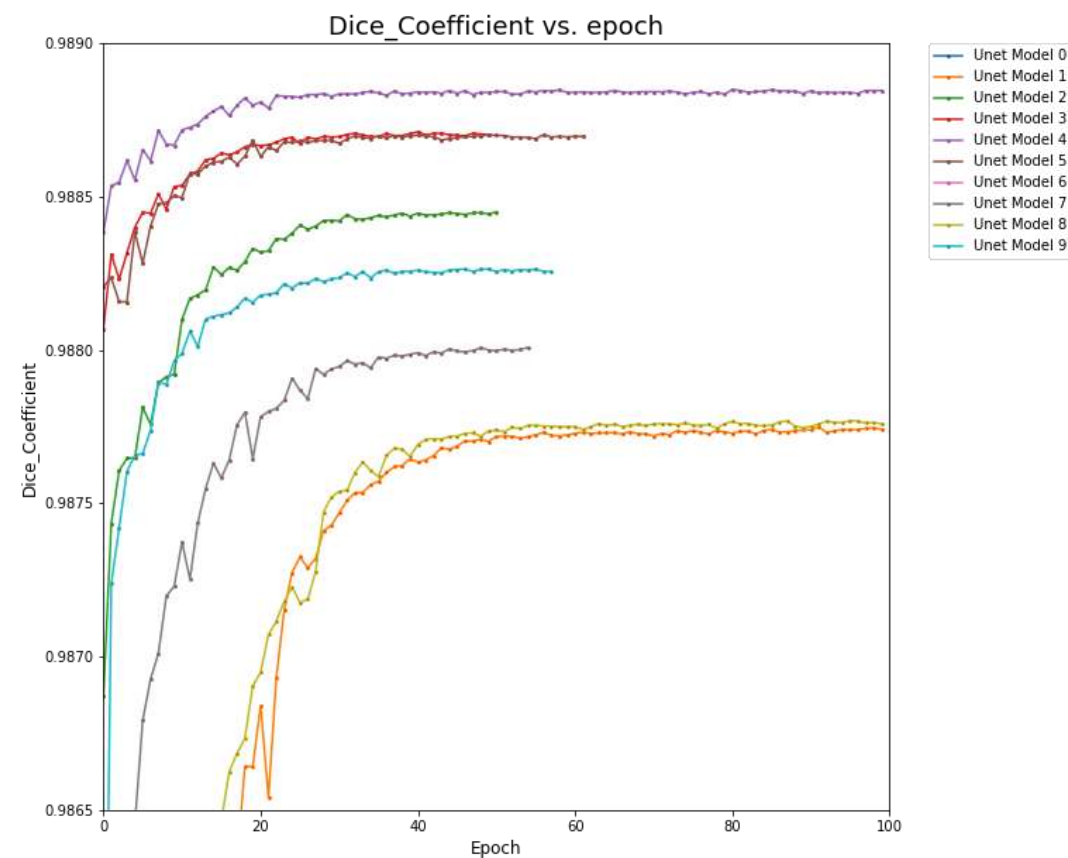


## Results and discussion

## Unet – Model V1



## Unet – Model V2



Results and discussion

Dice coefficient mean

Rat25 - UnetV2   Rat25 - UnetV1

Model		
0	0.923520	0.922404
1	0.921700	0.922108
2	0.921642	0.921837
3	0.921463	0.921766
4	0.921374	0.921595
5	0.924894	0.921351
6	0.922083	0.921658
7	0.921926	0.921755
8	0.921831	0.922874
9	0.921952	0.921971

Dice coefficient mean

Rat25 - UnetV2   Rat25 - UnetV1

mean	0.922238	0.921932
std	0.001108	0.000439
min	0.921374	0.921351
25%	0.921657	0.921682
50%	0.921878	0.921802
75%	0.922050	0.922074
max	0.924894	0.922874

Results and discussion

Dice coefficient mean

Rat25 - UnetV2   Rat25 - UnetV1

Model		
0	0.923520	0.922404
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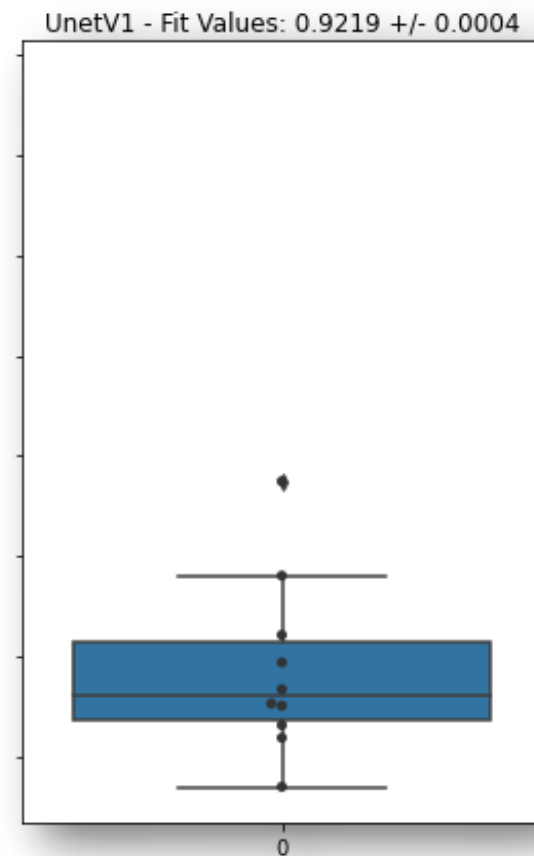
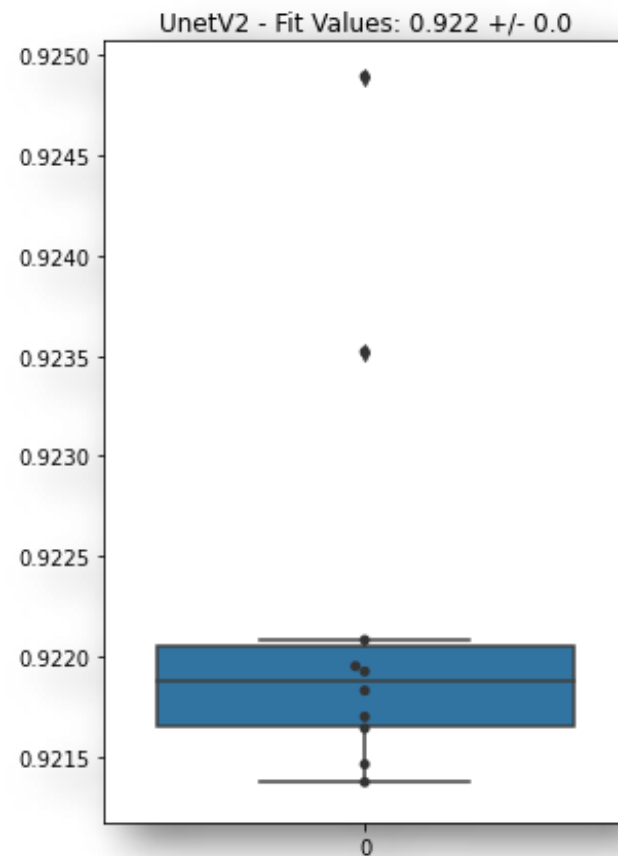
## Results and discussion

## Dice coefficient mean

Rat25 - UnetV2   Rat25 - UnetV1

## Model

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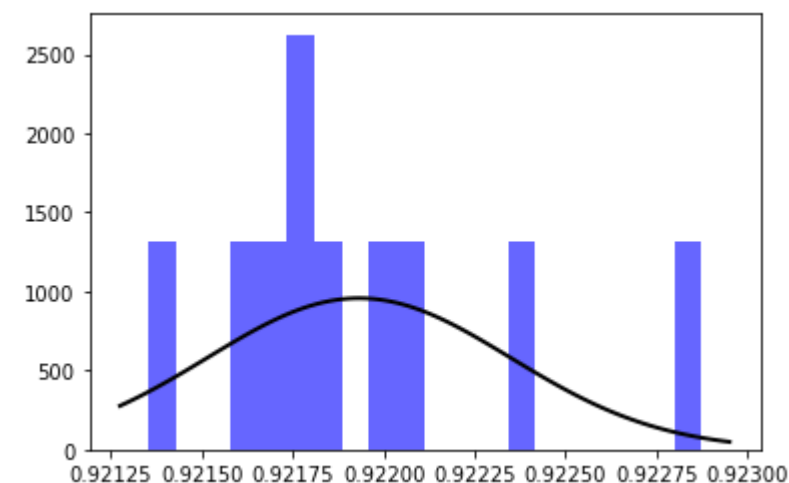
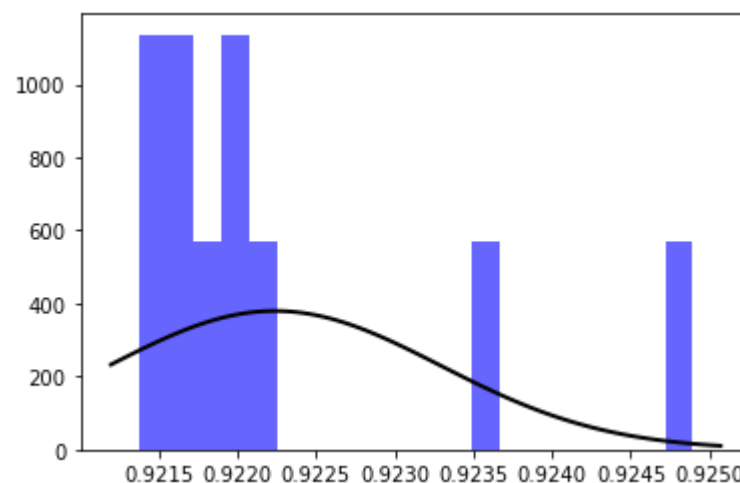
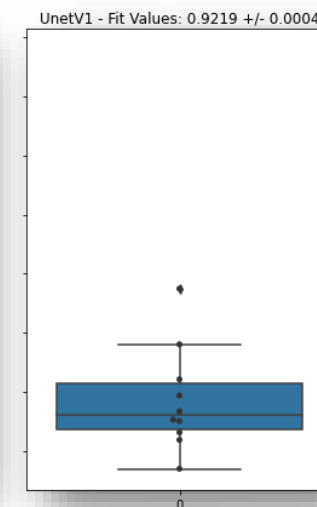
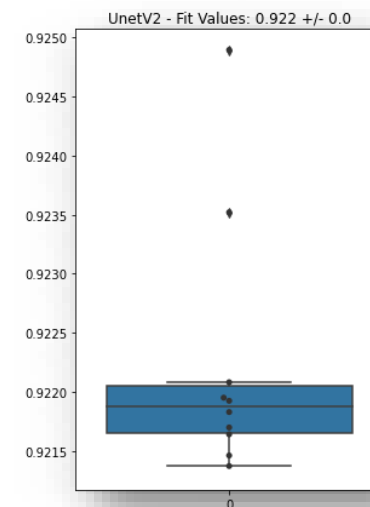
## Results and discussion

## Dice coefficient mean

Rat25 - UnetV2   Rat25 - UnetV1

Model

0	0.923520	0.922404
1	0.921700	0.922108
2	0.921642	0.921837
3	0.921463	0.921766
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## Wilcoxon Test

Perform the Mann-Whitney U rank test on two independent samples.

The Mann-Whitney U test is a nonparametric test of the null hypothesis that the distribution underlying sample x is the same as the distribution underlying sample y. It is often used as a test of difference in location between distributions.

Conditions:

- If  $P\text{-value} < 0.05$ : Reject NULL hypothesis - Significant differences exist between groups;
- If  $P\text{-value} > 0.05$ : Accept NULL hypothesis - No significant difference between groups.

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Conditions:

Dice Coefficient:

H-statistic: 49.0

P-Value: 0.9698499769931556



- If P-value < 0.05: Reject NULL hypothesis - Significant differences exist between groups;
- If P-value > 0.05: Accept NULL hypothesis - No significant difference between groups.

## Futures Objectives

## Deep RNAs applied to Rats tibia



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