Quantitative Morphometry in Arthritis Diseases

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The scope of my thesis

In the development of drugs for Osteoarthritis, some of the research includes test on mice which spontaneously develop the desease. To determine the level of osteoarthritis each mice is given a score manually this is time consuming and suffers from bias. The scope of this thesis is to derive quantitative measurements using image analysis to describe the joint degradation in the mouse model using the medial tibia cartilage. This is done in collaboration with Visiopharm

About Visiopharm

Visiopharm is a Danish company that develop software components used in microscopes for image acquisition, advanced image analysis, stereology, automation, data management, and reporting. Visiopharms system is used in biopharmaceutical companies, universities and hospitals.

Osteoarthritis

The Disease Osteoarthritis is the most common type of arthritis. Especially older people is a subject to the disease, that typically strikes in the weight-bearing joint of the patient and leads to pain and loss of mobility.
Almost everybody above the age of 60 has osteoarthritis in at least one joint.

Staining Methods

To be able to distinguish different tissue types in the mouse knee it is stained. There are three types of staining used in this project. They are:
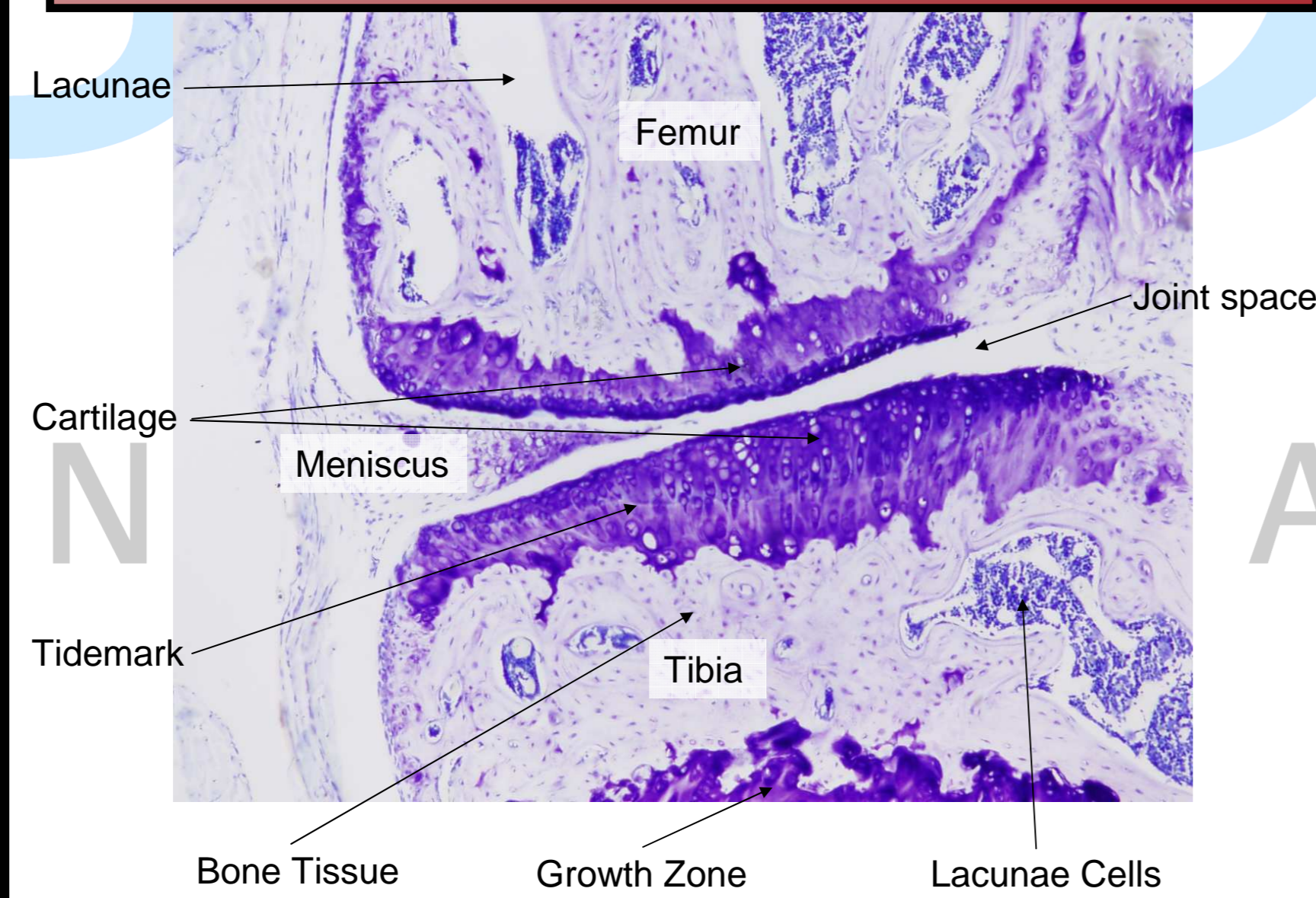
- Haematoxilin/Eosin
- SafraninO
- Toluidine Blue

They all have there advantages and there drawbacks, but the toluidine blue one is the fastes and easiest to stain in the lab. Examples of the staining can be seen in the colon to the right
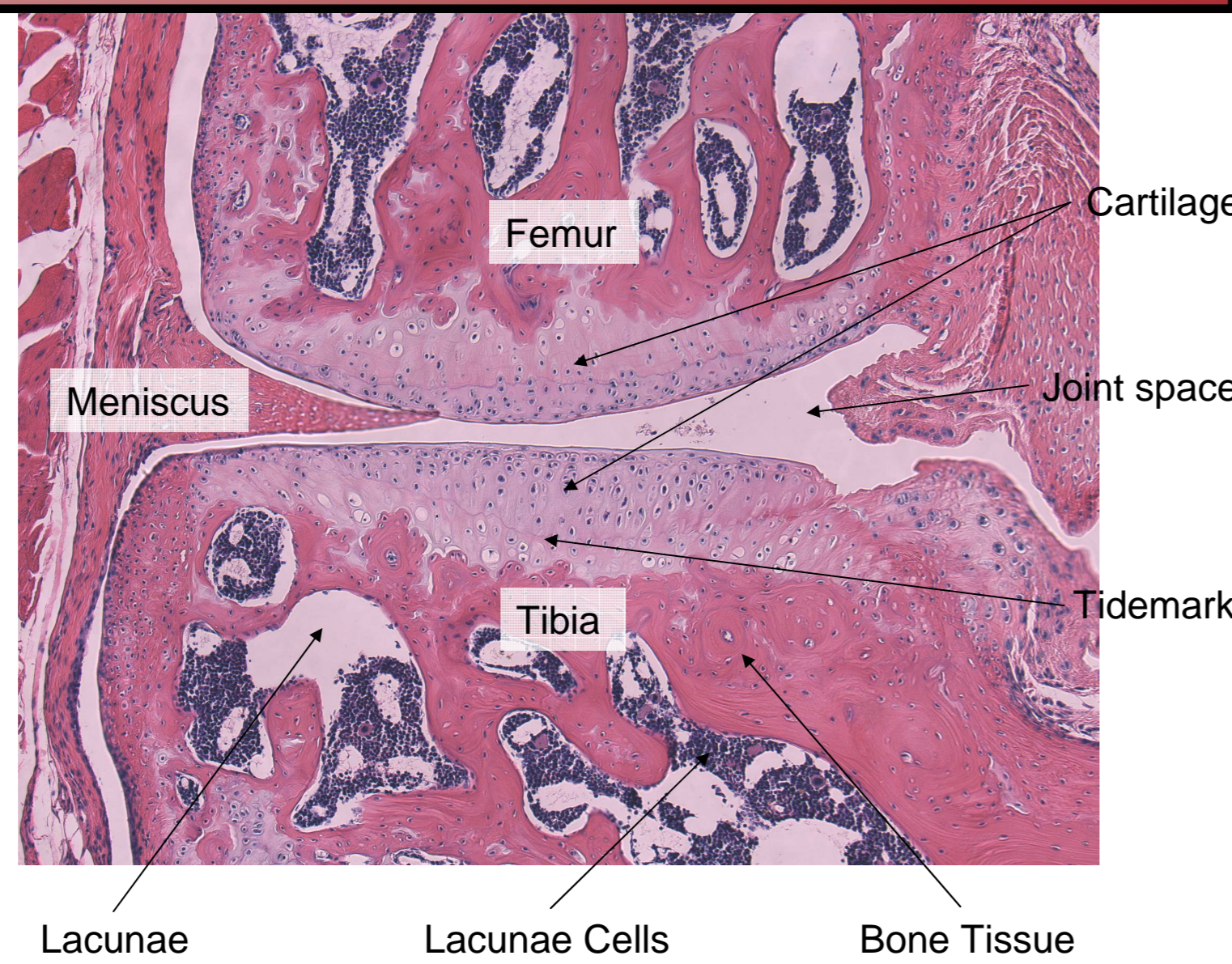
Images of mouse knees

To give the reader some idea of the images and the staining methods used in this project three exaples have been illustrated and areas of interest have been labeled.
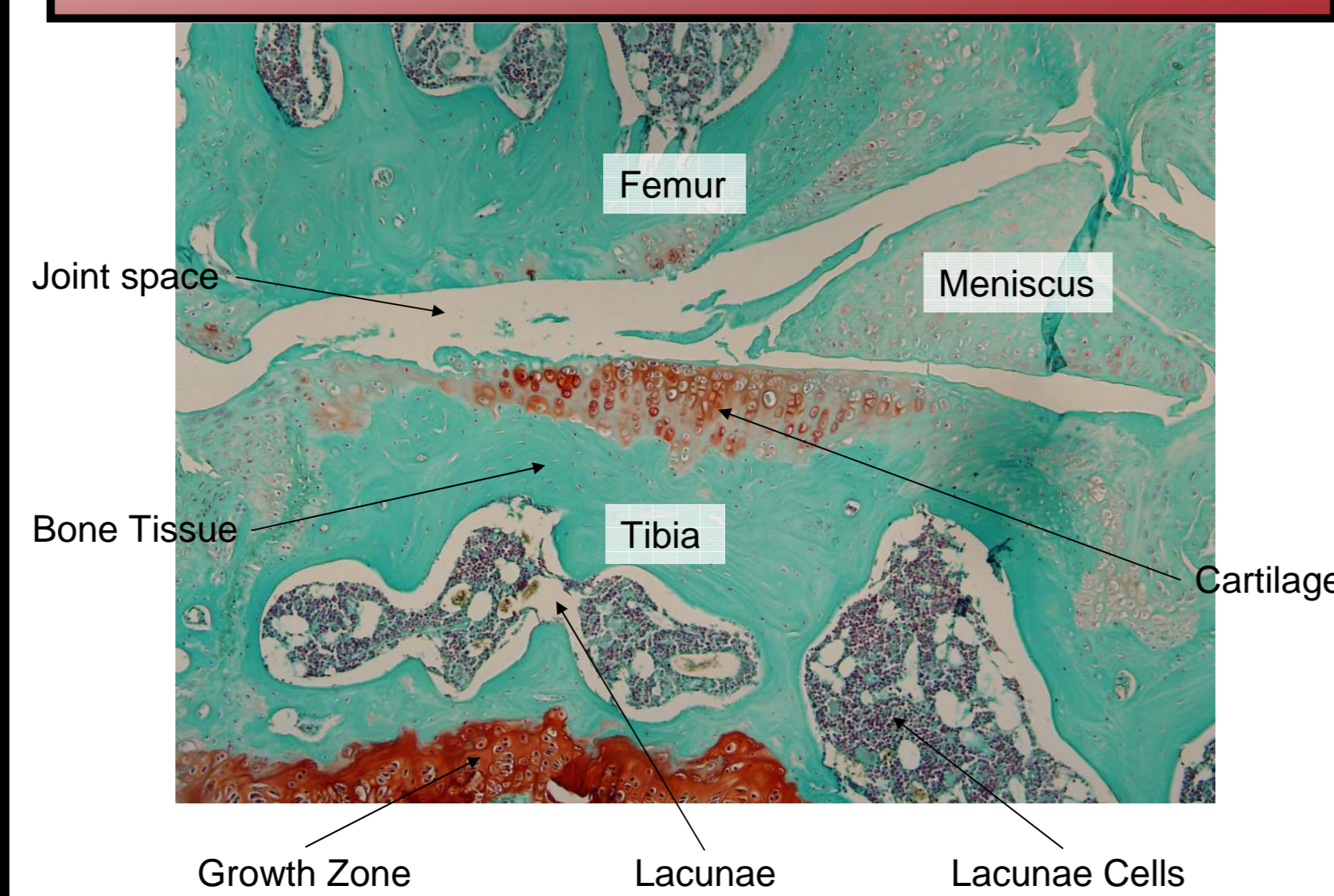
### Toluidine Blue

Haematoxilin/Eosin

SafraninO

Segmentation

Visiopharm Already have software to segment Haematoxilin/Eosin and safraninO stained images, and for these two staining method they can semi automatic collect quantitative mesurements, which describes if the mouse have osteoarthritis or not. So the first task was to segment the toluidine blue images. Since this project only involves the medial tibia part of the knee that is what is segment.

The first part of the segmentation is a threshold of the green and red band to find the growth zone and medial tibia cartilage. A 8-connectivity element is used to find connected pixel.
Using least square regression on all the pixels found to be cartilage the image is rotated so that the cartilage is horizontal.
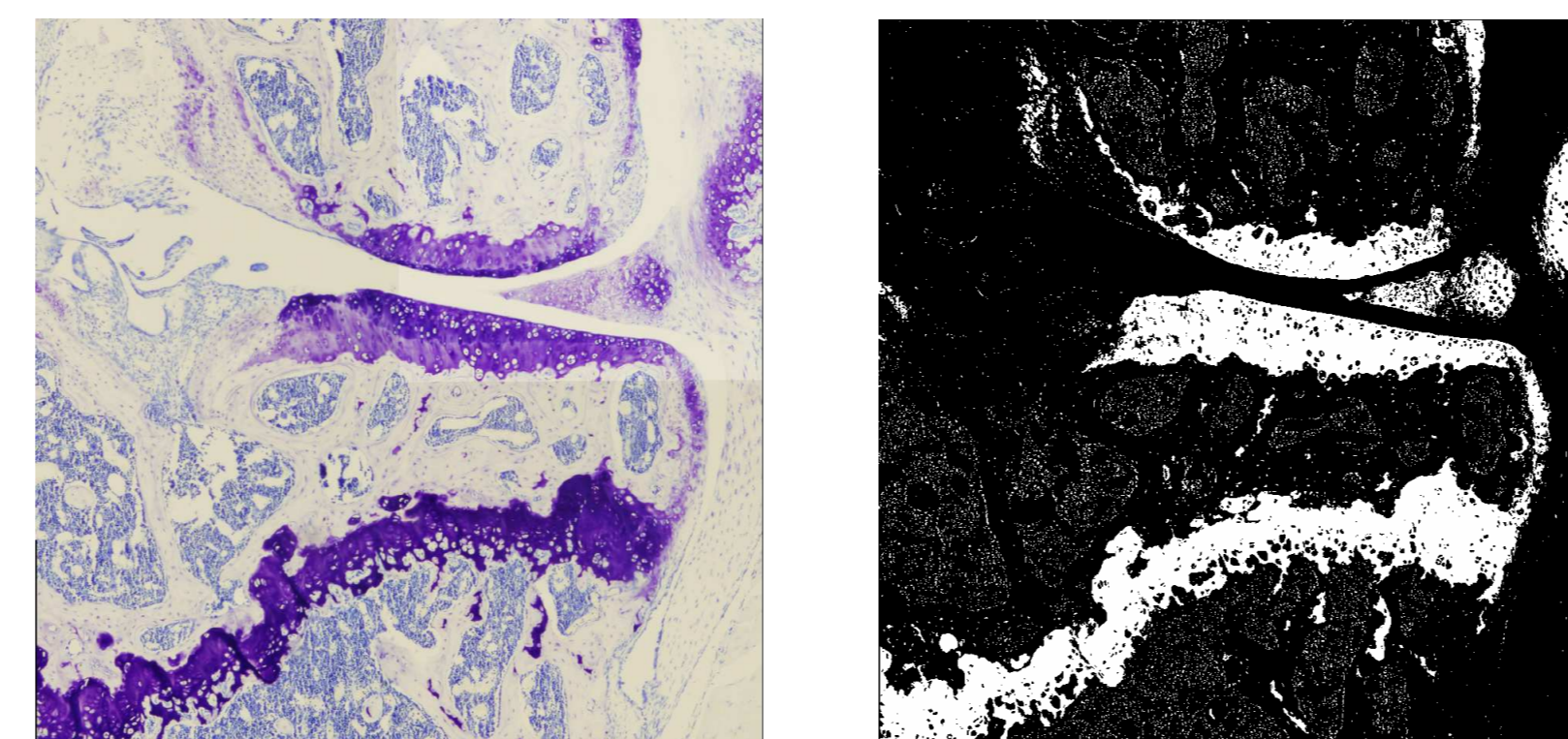

In the area between the cartilage and the growth zone all the pixels is first defined as bone and then thresholdet to find lacunae and then the lacunae is thresholdet to find the lacunae cells.
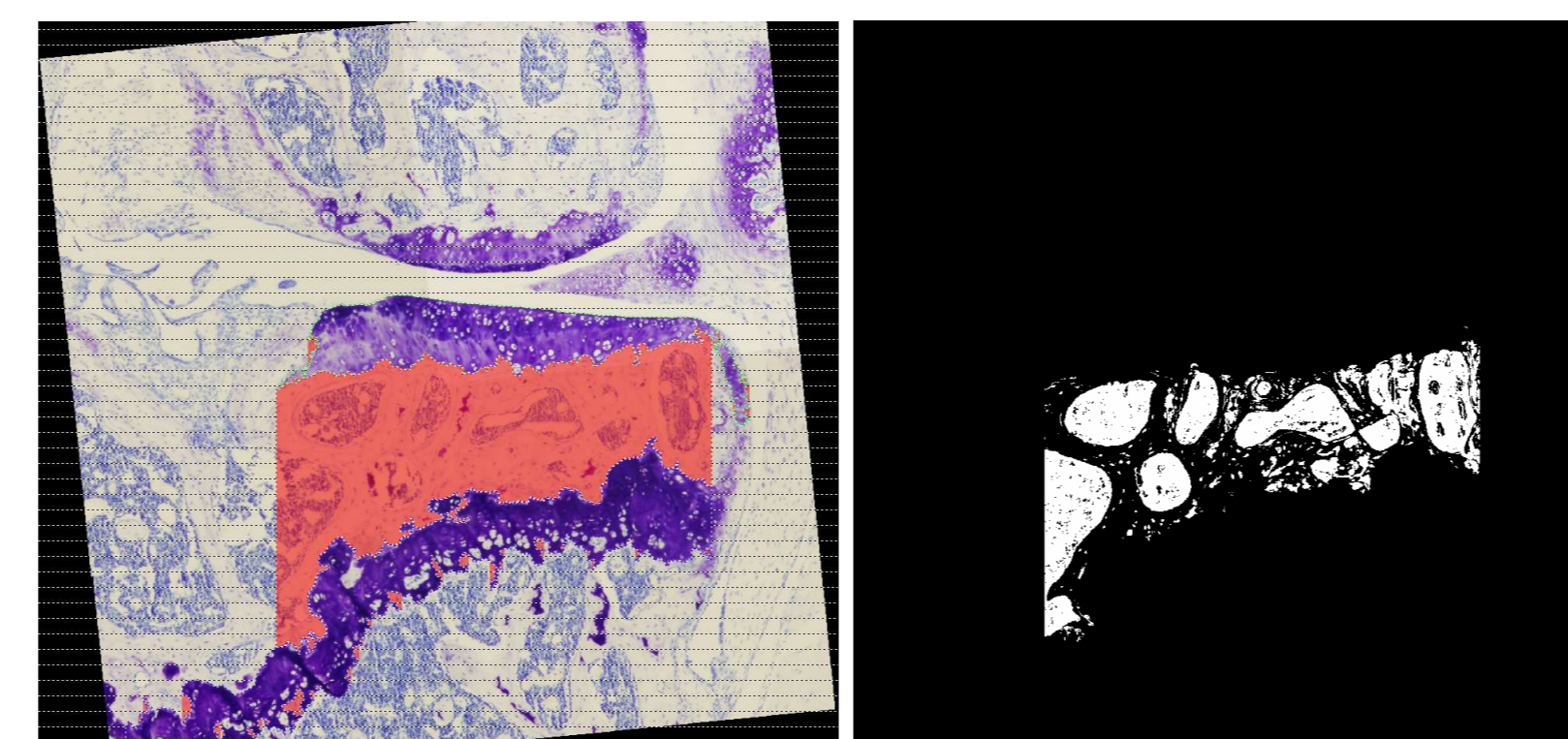
The next thing is to cut off the cartilage in both sides to remove areas with little or no information. This cut off happens when the cartilage thickness is below 20% of the thickness in the middle of the cartilage or if the thickness of the cartilage suddenly drops more than 50%. These stopping criteria's is found by test.

The next part of the segmentation is to find the tidemark, the tidemark is a line that runs between articular cartilage and calcified cartilage. It is important to find this line because you only want to make measurements in the articular cartilage. As it can be seen on the labeled images to the left the tidemark is difficult to see.
In this project dynamic programming is used to find the tidemark, (Dynamic programming is a method that finds the darkest line running thru the image) but some preprocessing steps is nessesary to make this method work. The first step is to remove the background so that the dynamic programming only can run in the cartilage. The next step is to define start and end points. This is done by taking the cut off described earlyer and move 20 pixels down from the top and use these points. To make the tidemark apear as the darkest line in the image the colors are negated and a blobfilter is used to remove the, now very dark cells in the cartilage futher the colorshift from bluish to purple is taken into account.
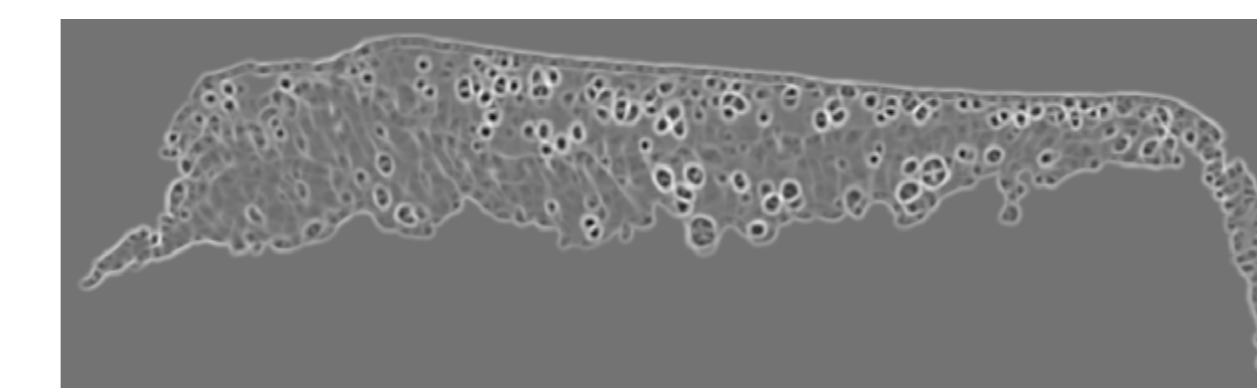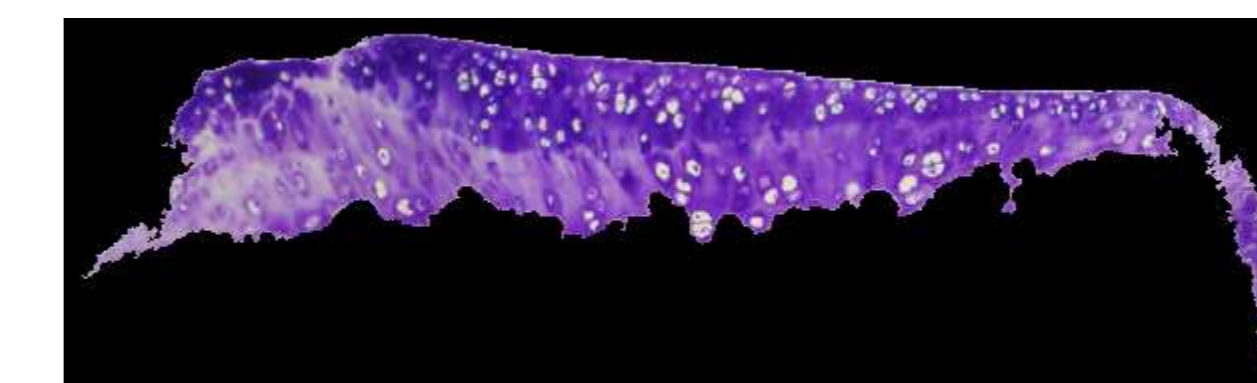
After the tidemark is found the articular cartilage is split up into three different parts so that they can be analysed seperatly.
The segmentation should make it possible to determine if there is any damage to the articular cartilage and if there damage then determine how severe it is. The final result of the segmentation can be seen in the next colon.

Final Results

The segmentation makes it possible to take measurement which in the end should lead to end points that can tell if the mouse have osteoarthritis or not.
The things that should be measured, is the colour intensity of the articular cartilage, the fibrillation which is a measure for how much of the articular cartilage is missing, the amount of dead cells in the articular cartilage and the thickening of the subchrondral bone which is a measure of the bone / lacunae density between the cartilage and the growth zone.
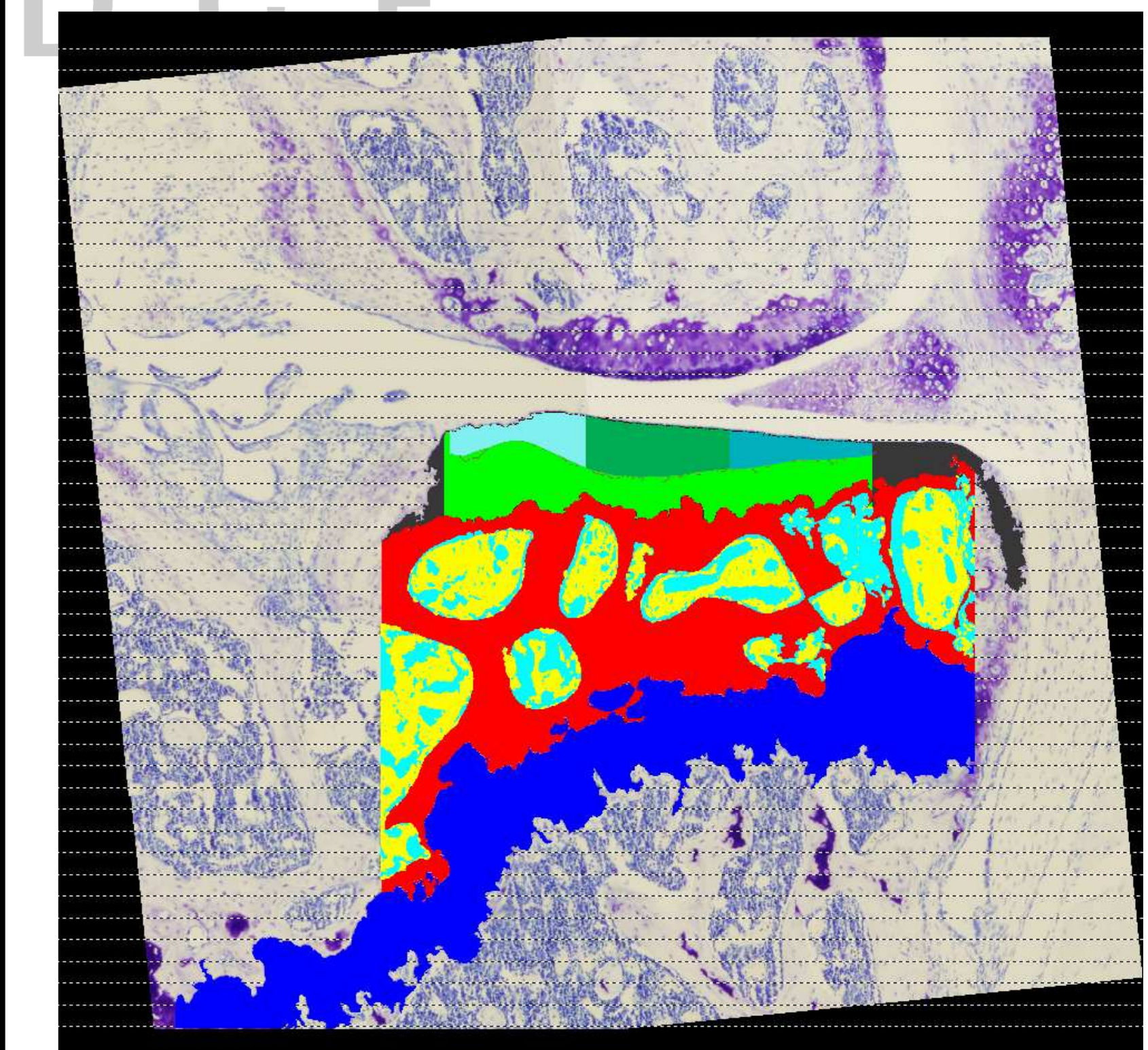
Final segmentation of the toluidine blue image. Blue is the Growth Zone, Turquoise is the Lacunae, Yellow is the Lacunae Cells, Green is calcified cartilage, black is the parts of the cartilage that is cut off and the 3 areas in the top of the cartilage is articular cartilage.

Further Work

My work on this thesis is not yet finish, so further work is what I need to do before it is done. The first thing I should do, is to make the measurement and correlate them with the manual scores.
If this works the preprocessing to the dynamic programming should be made so that it works on the Haematoxylin/Eosin and SafraninO stained images. At the moment the user draw the tidemark themselves and the cut offs in the side is also done manually.
So a set of cut off criteria's should also be found for these two staining methods.