



# Systemic granulomatosis in meagre

## A presentation full of disgusting pictures

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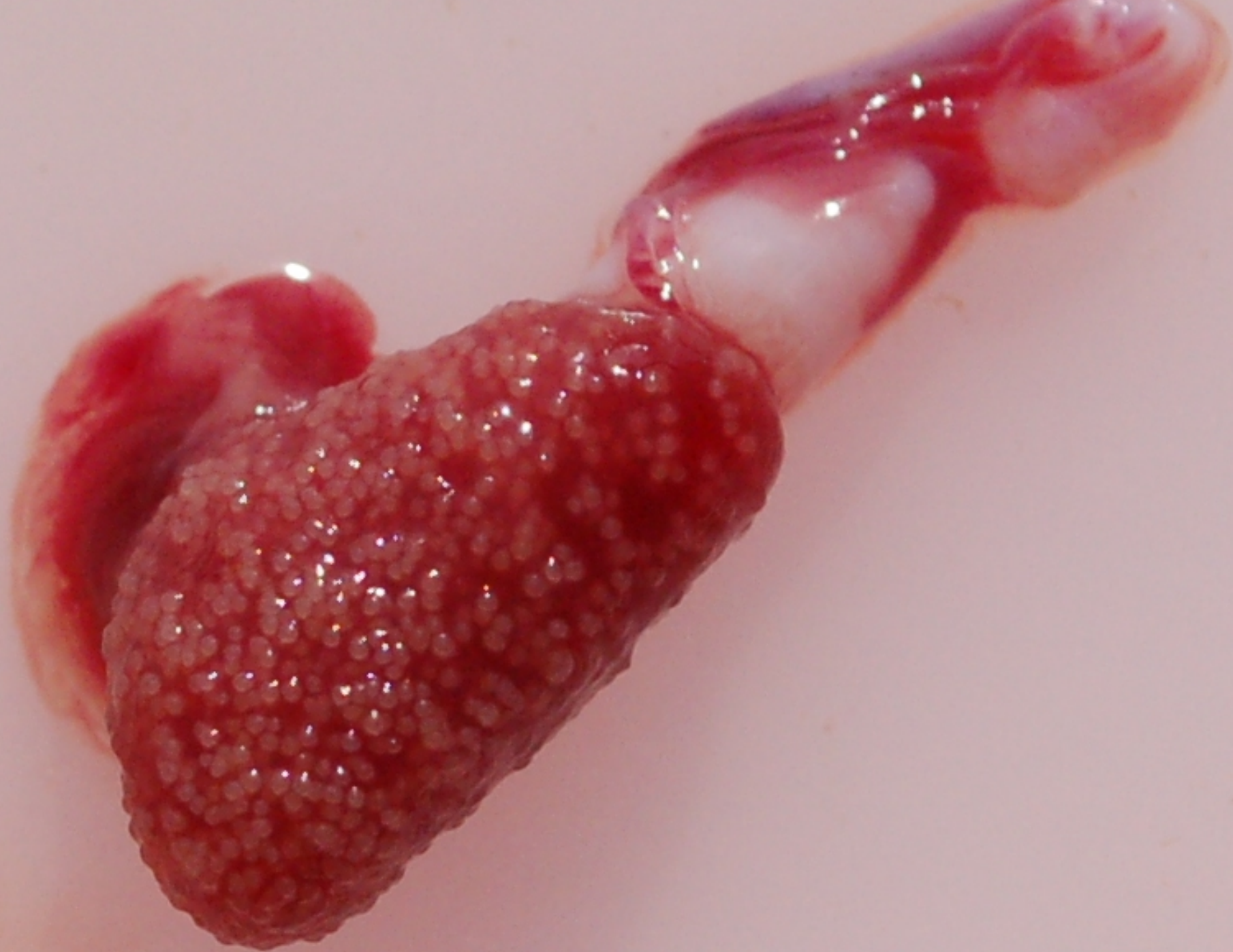
**Barcelona, 18 Jan 2017**

# Systemic granulomatosis

- Bottleneck for meagre production
- Disease of unknown aetiology
- Affects 100% of the population
- Severity: from very mild (undetected) to.....you will see
- Major task for WP24

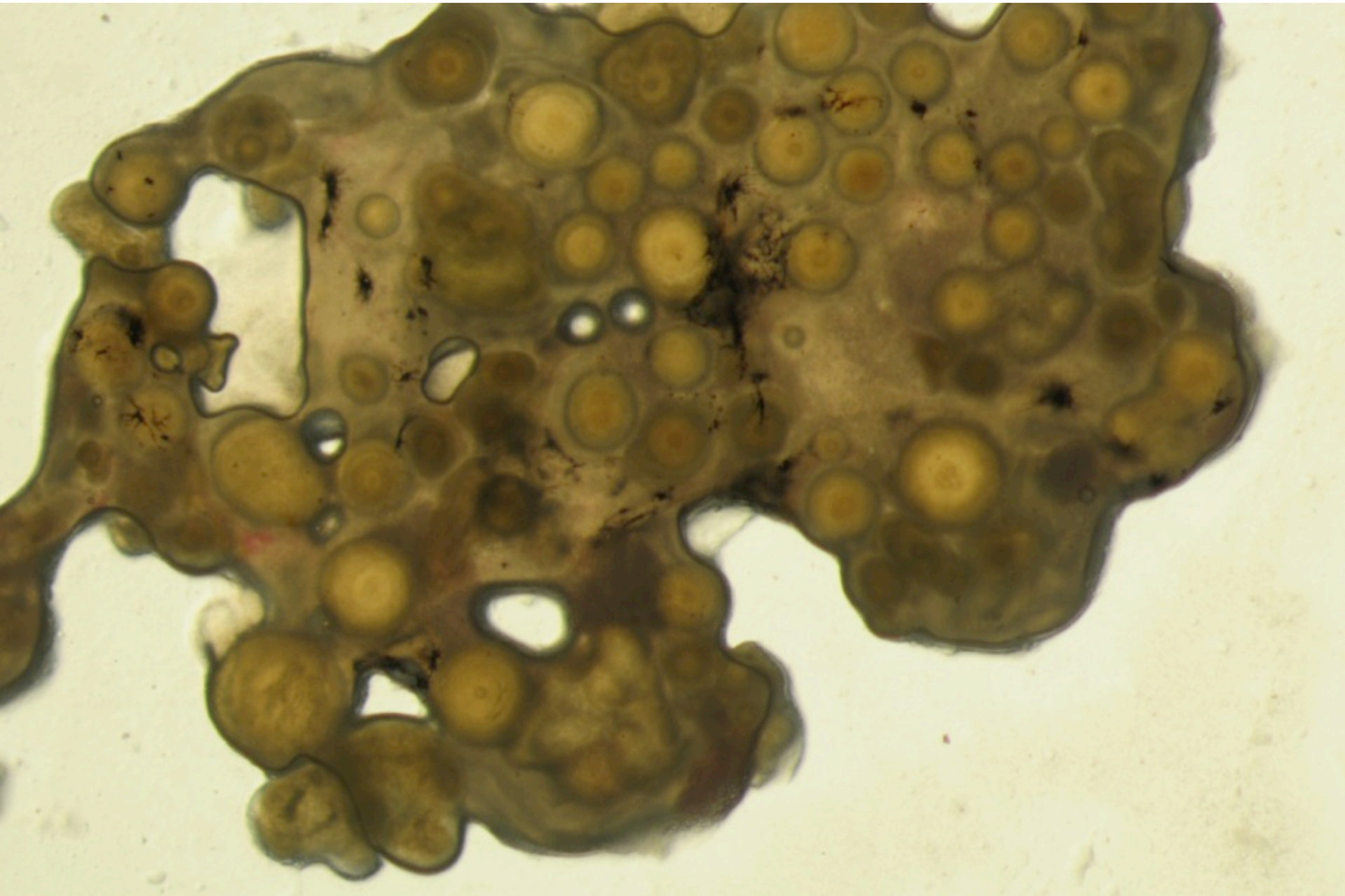




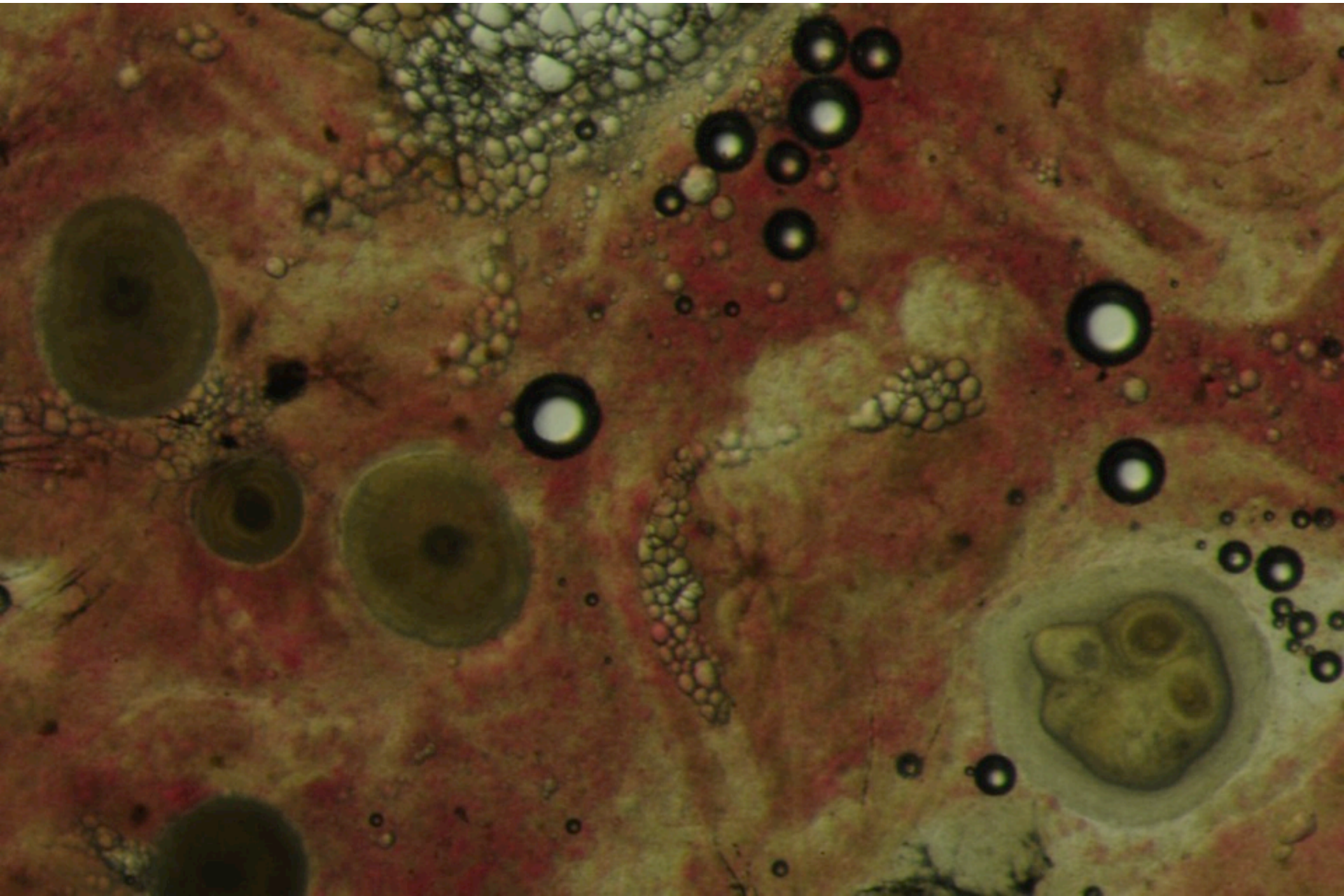


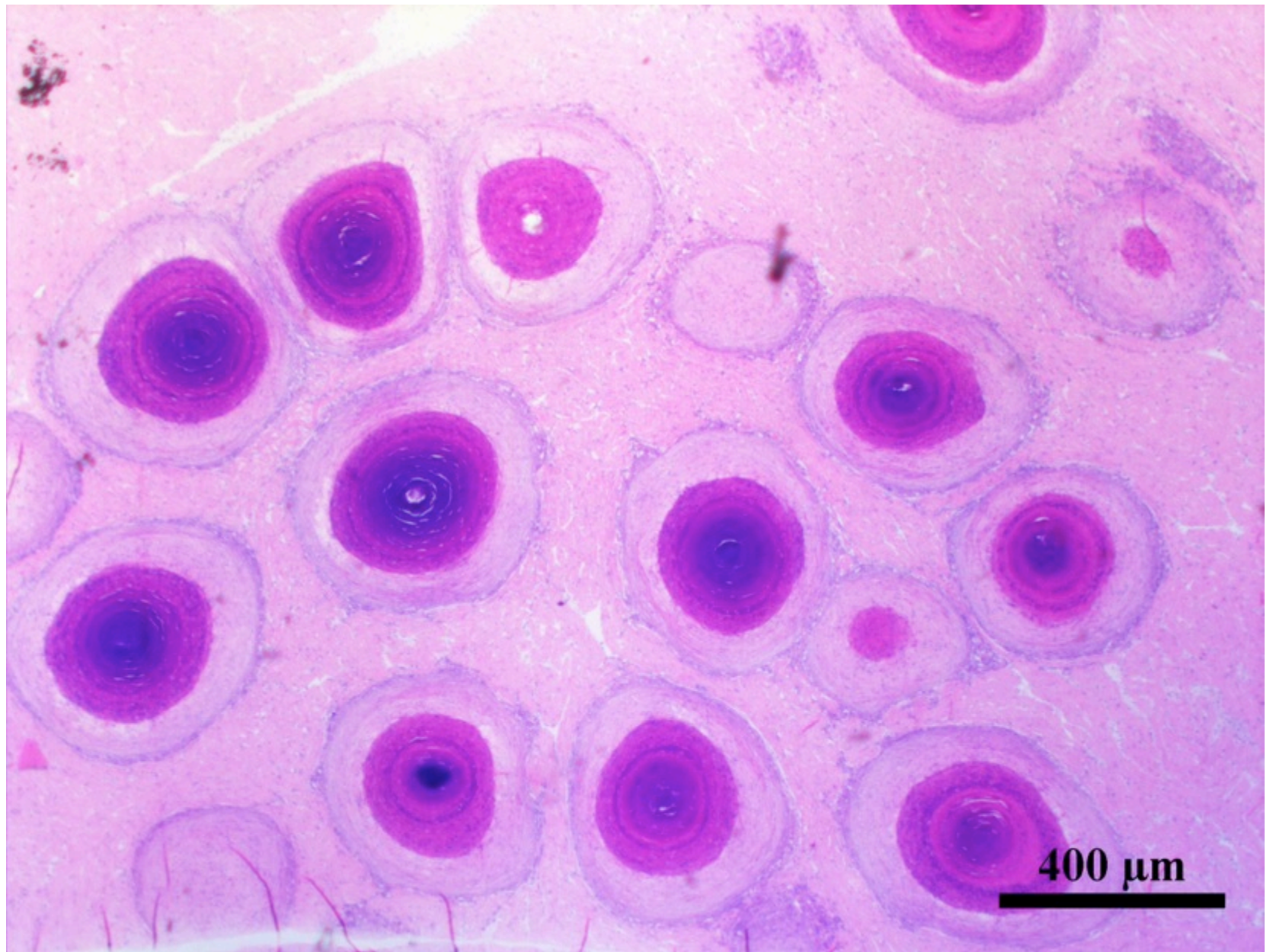


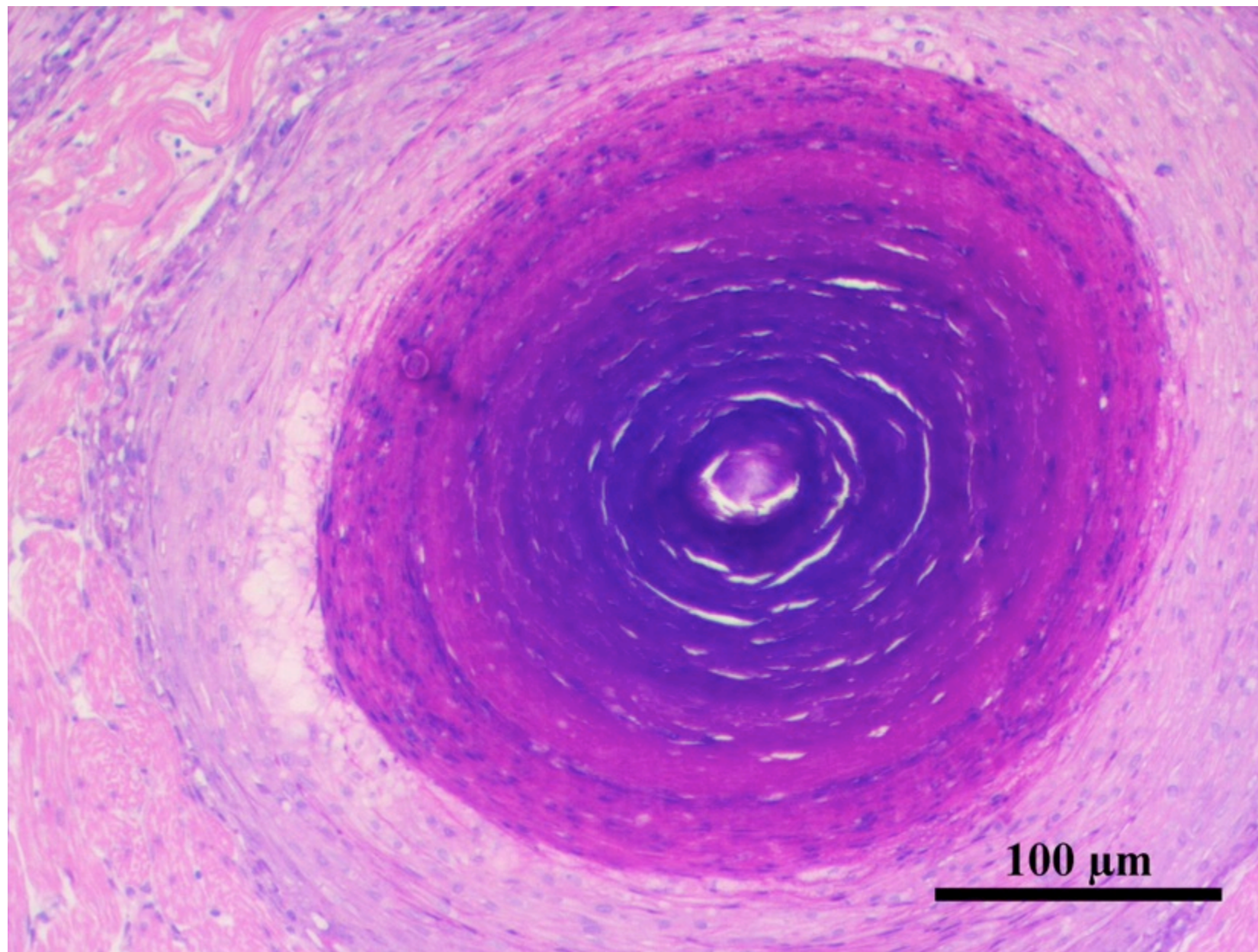




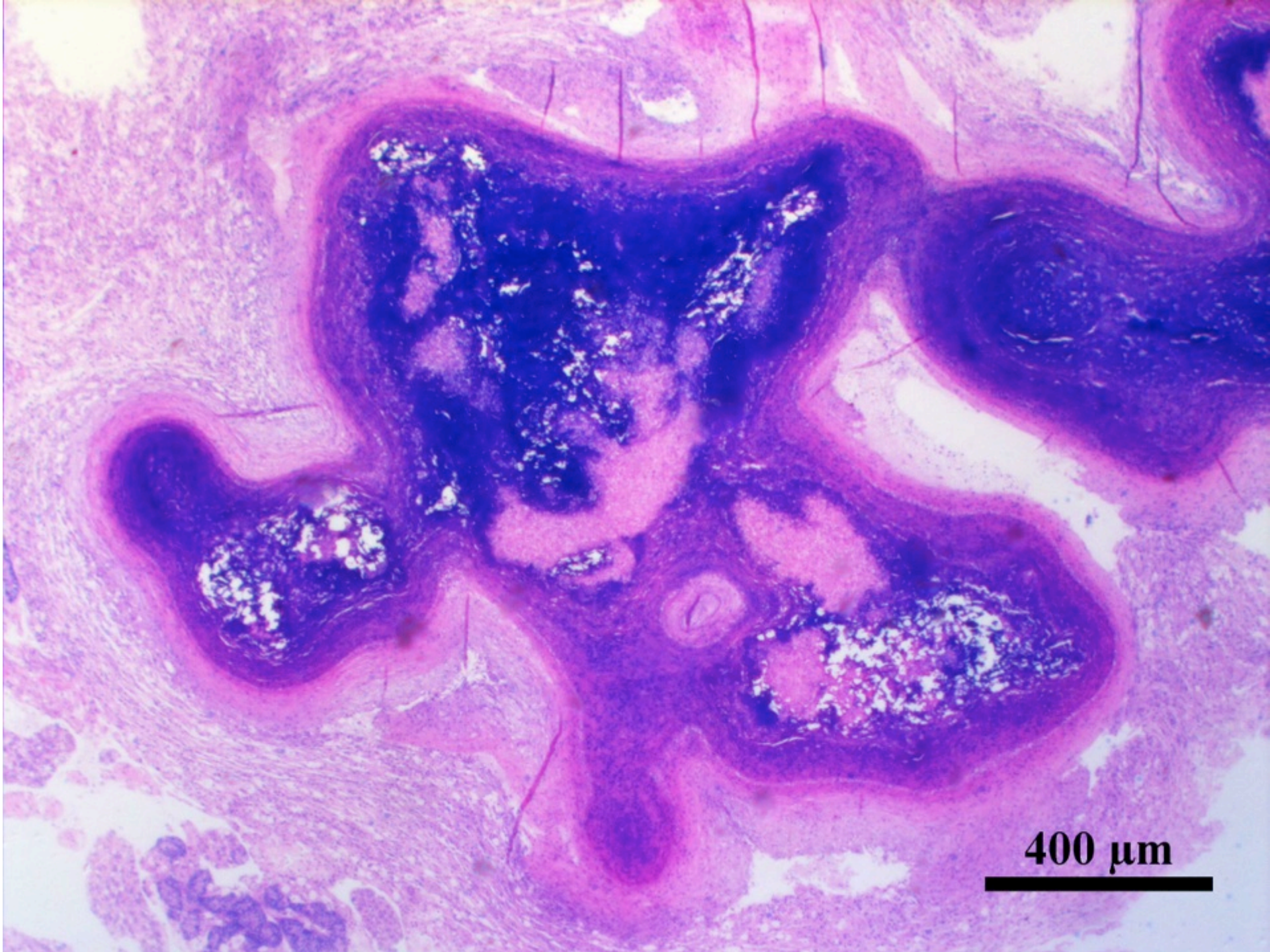




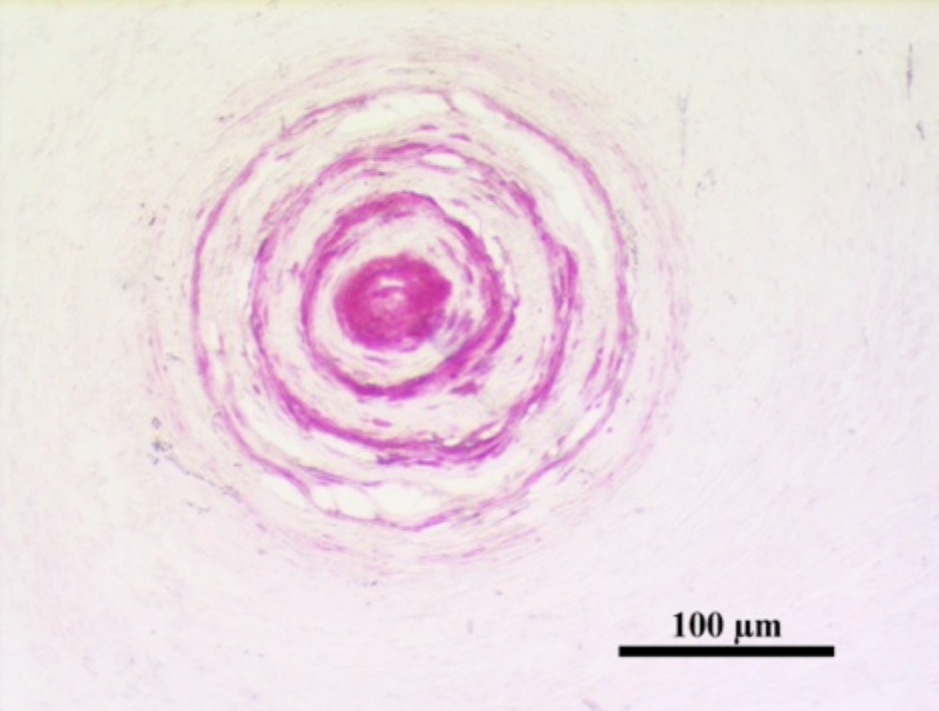




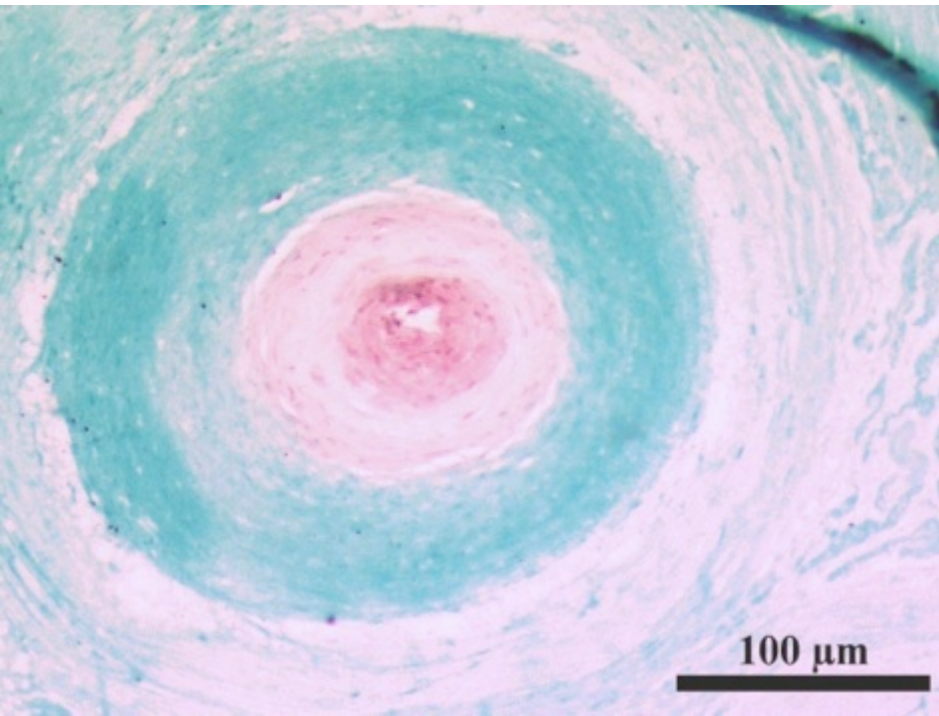
100  $\mu\text{m}$



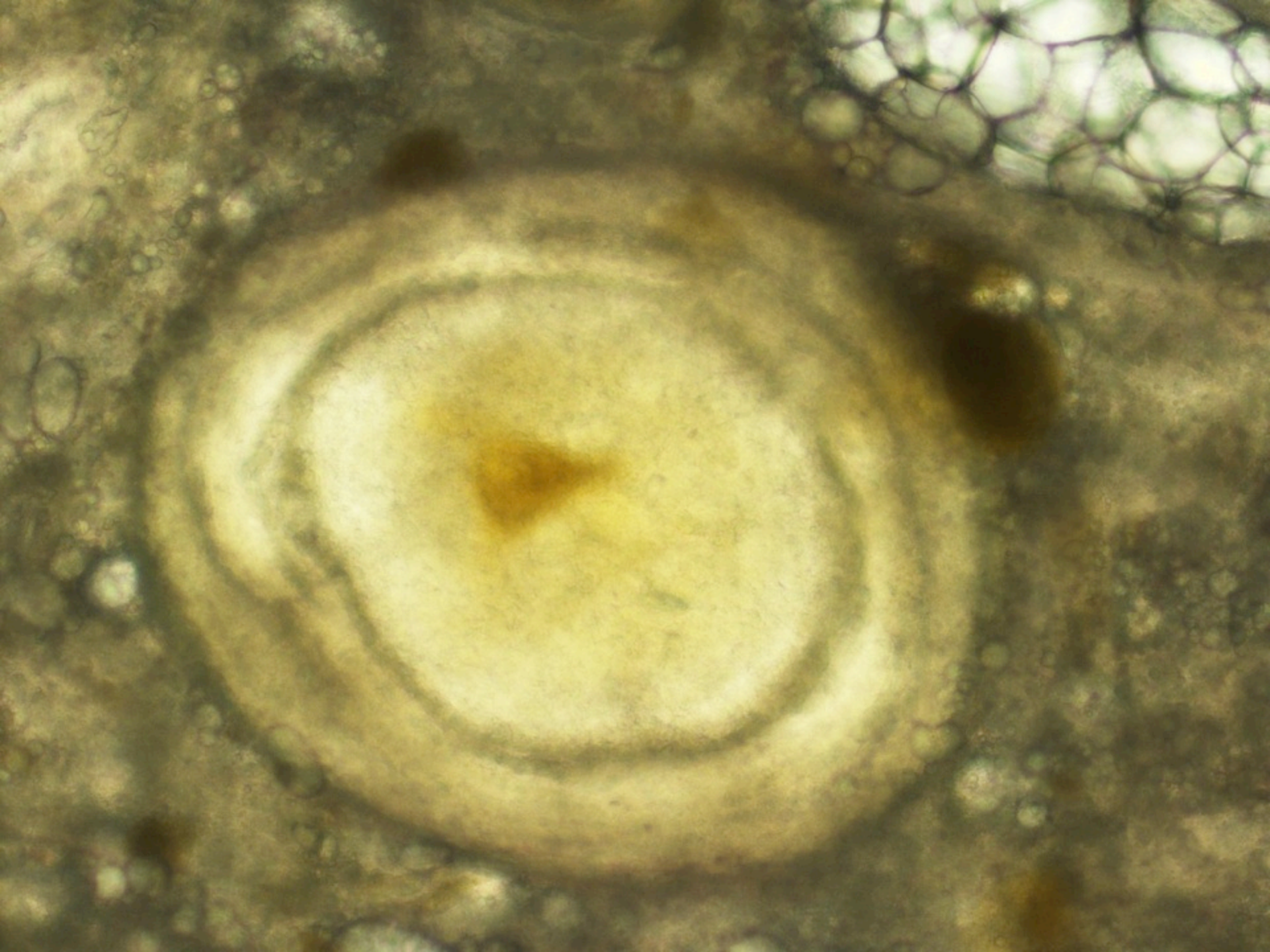
400  $\mu\text{m}$

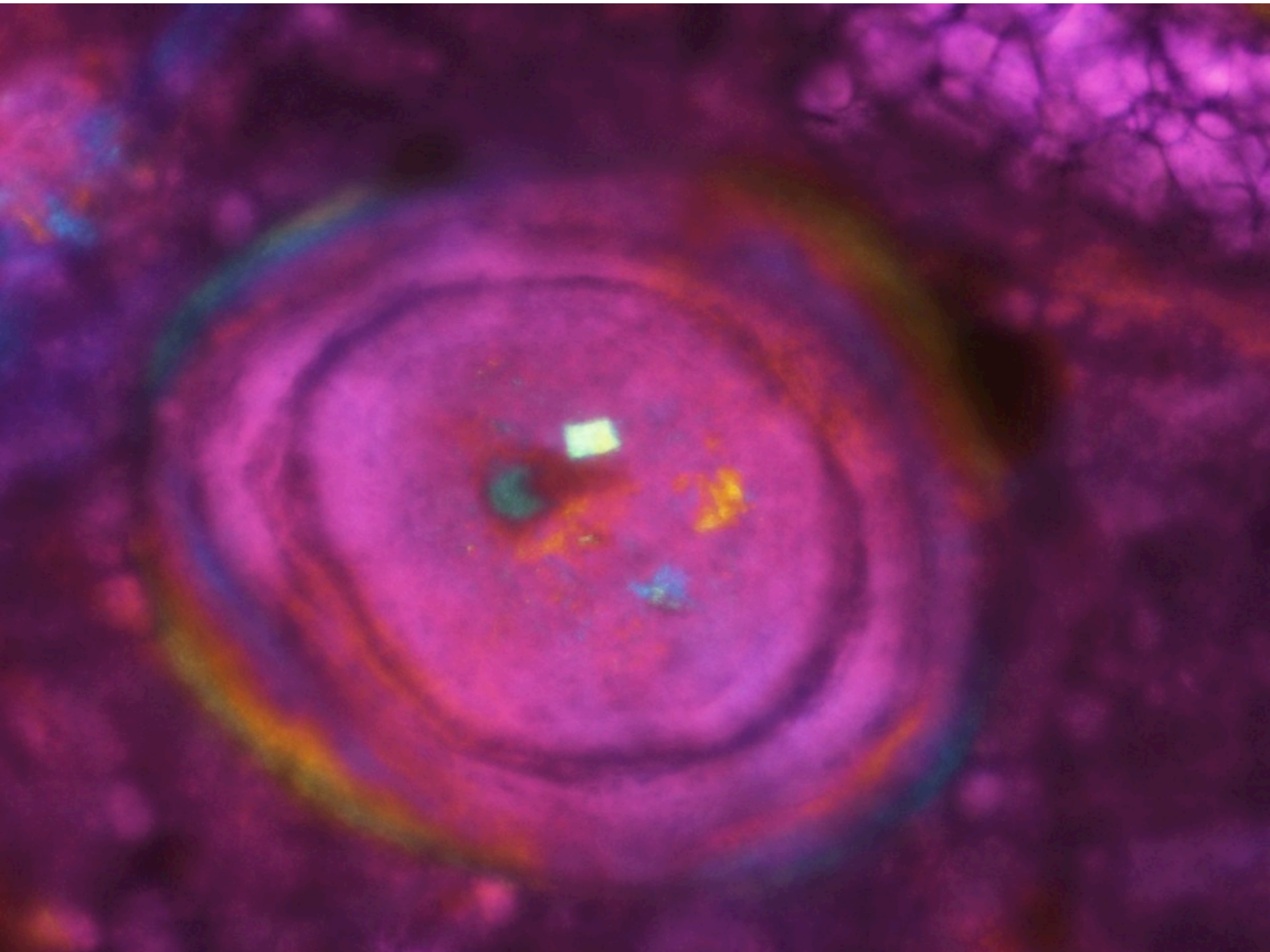


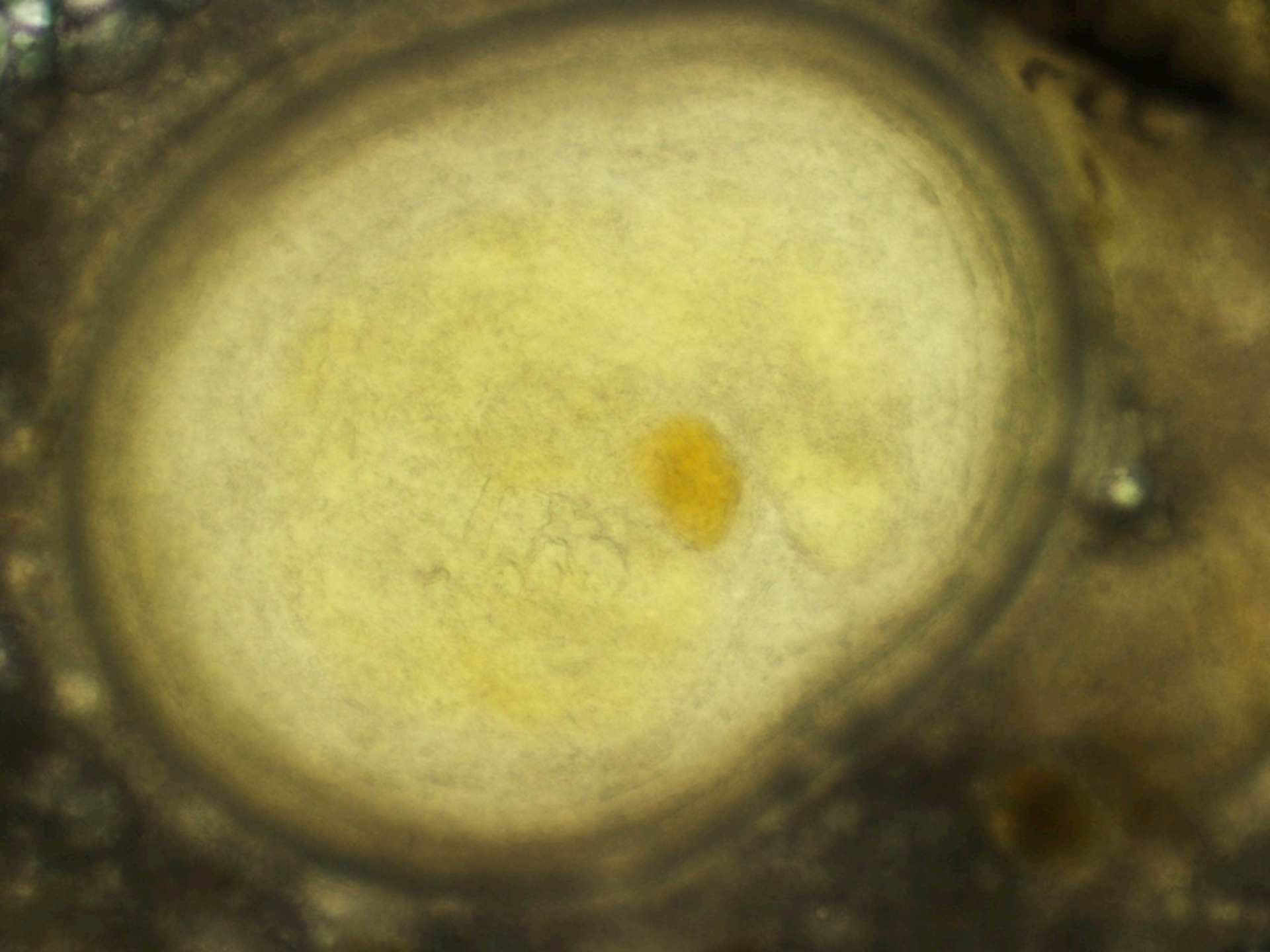
Alizarin stain



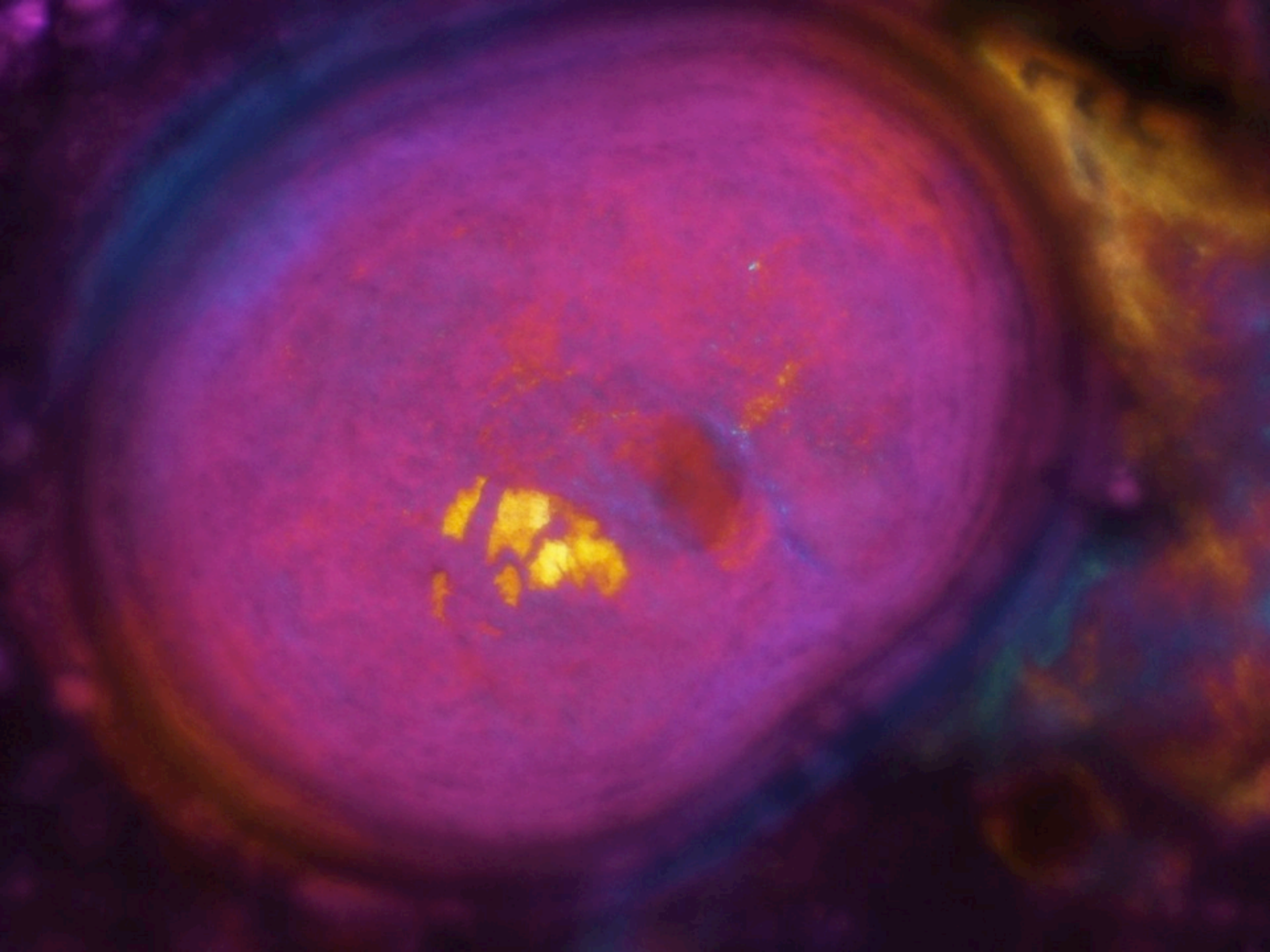
Alizarin stain  
counterstained with Fast green

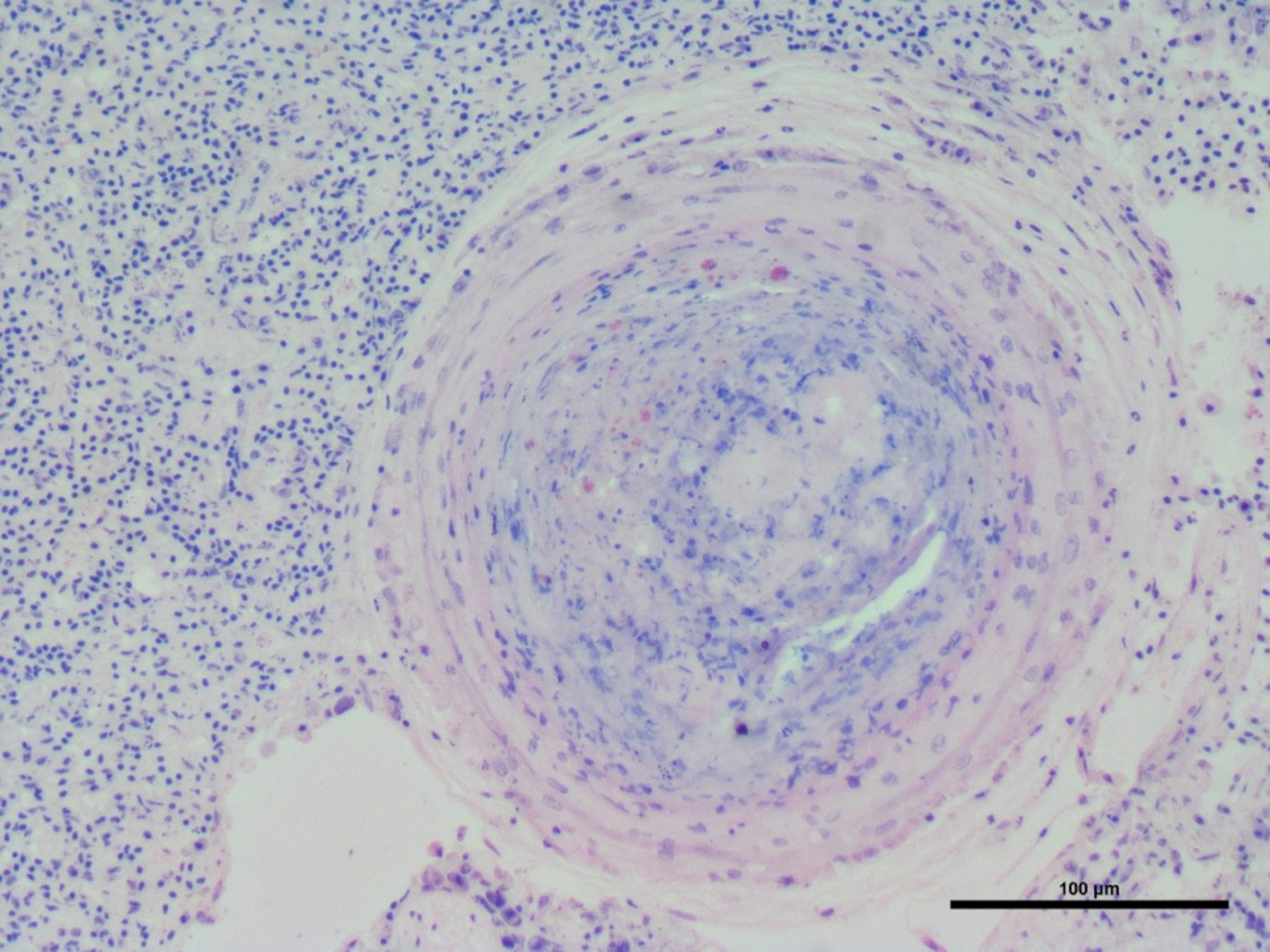












100  $\mu$ m



# Hypotheses

## **Nutritional (metabolic?) disease**

- Visceral granulomas in sea bream-hypertyrosinaemia
- Visceral granulomas in brook trout
- Renal granulomas in turbot
- Some reports in cichlids and goldfish

## **Disease caused by pathogens**

- Nocardia/mycobacteria
- Other granuloma-inducing pathogens (fungi, bacteria, intracellular parasites...)

# DIVERSIFY WP24

## feeding trials

### HCMR

- Effect of Vitamin D
- Effect of Ca/P ratio
- Effect of plant proteins

### FCPCT

- combined effect of vitamins E, C and carotenoids
- effect of Se, Mn and Fe

# The effect of vitamin D

# *Experimental design*

July 2014 – October 2014

4 diets

Diet	IU/Kg
D0	4550
D1	7000
D2	10000
D3	20000

12 tanks (500 l)

600 fish (Initial weight ~ 4g)

# *Experimental design*

## **Samplings every month**

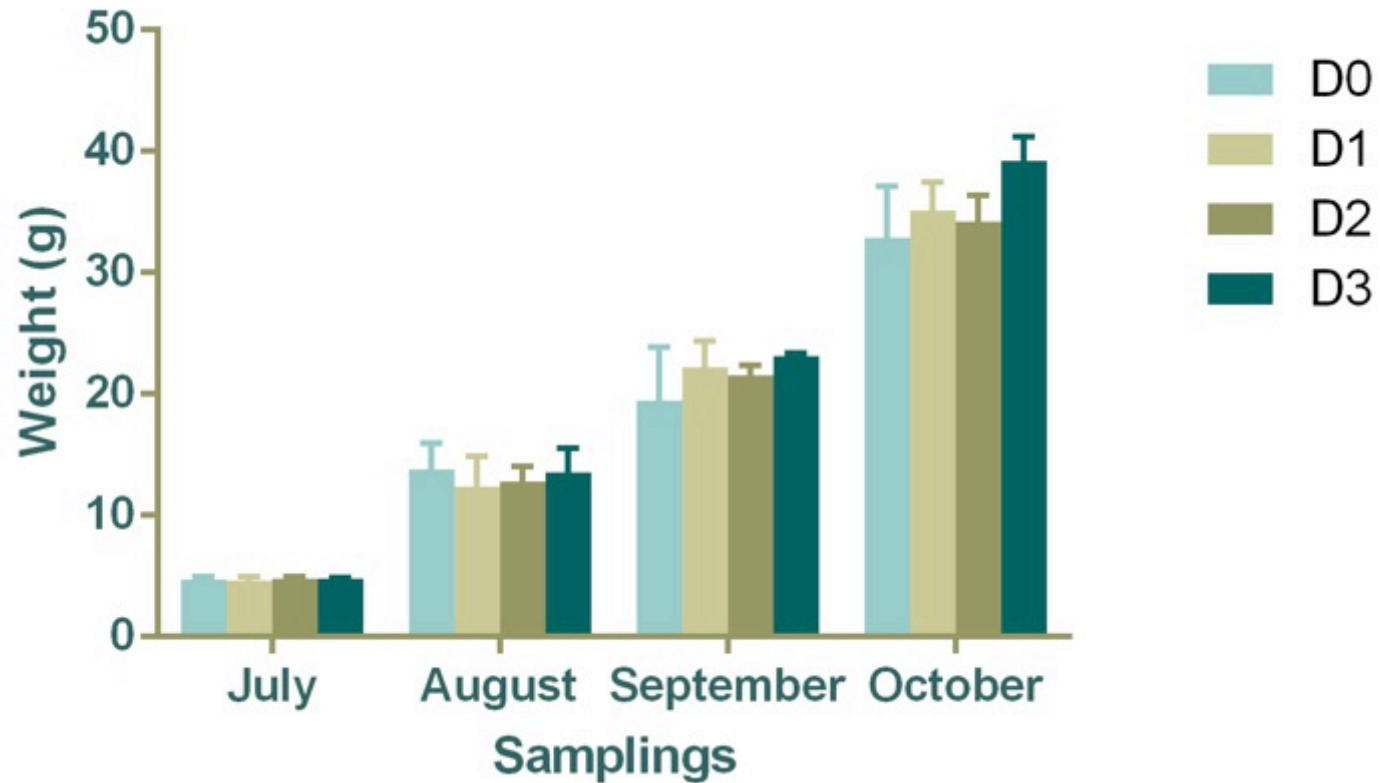
- Growth performance
- Evaluation of granulomas
- Histology
- Plasma analysis

<b>Score 0</b>	<b>No granulomas</b>
<b>Score 1</b>	Granulomas visible only with microscopy
<b>Score 2</b>	Granulomas visible macroscopically
<b>Score 5</b>	Tissue calcification



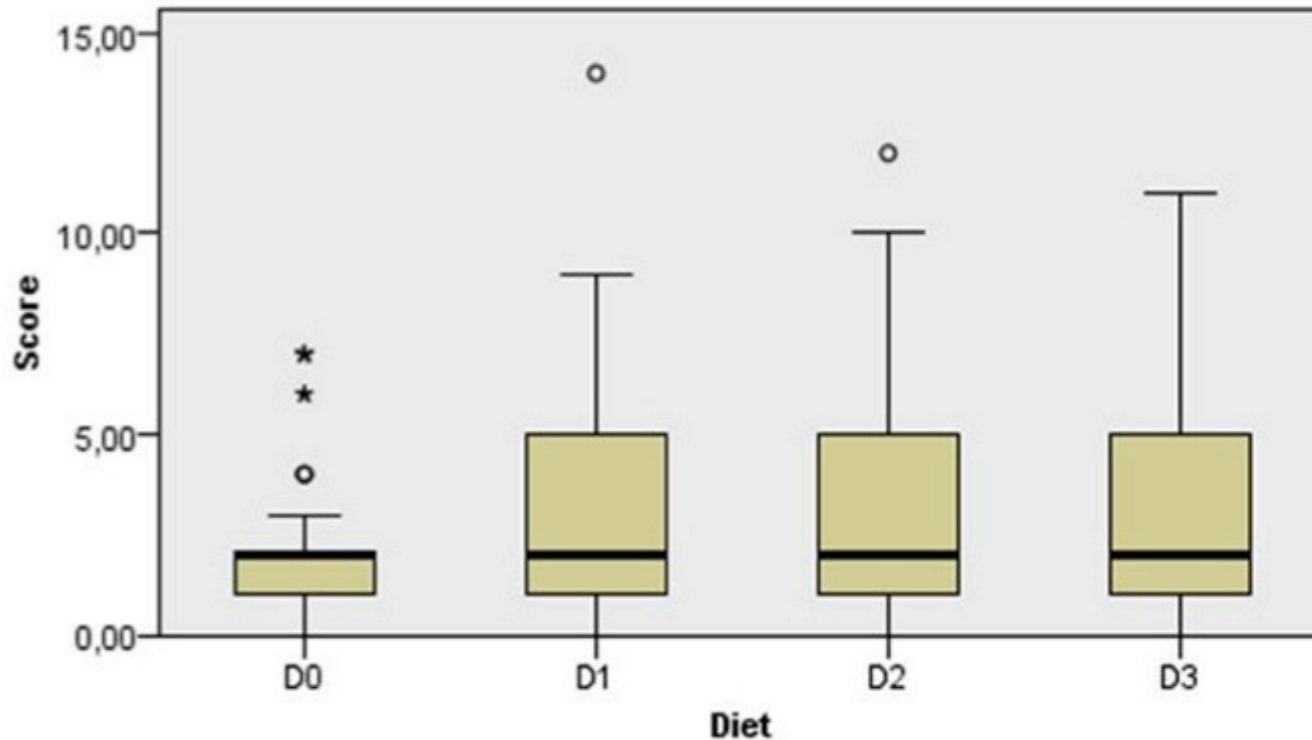
# Growth performance

- Juvenile meagre grew from ~4 g to ~35 g



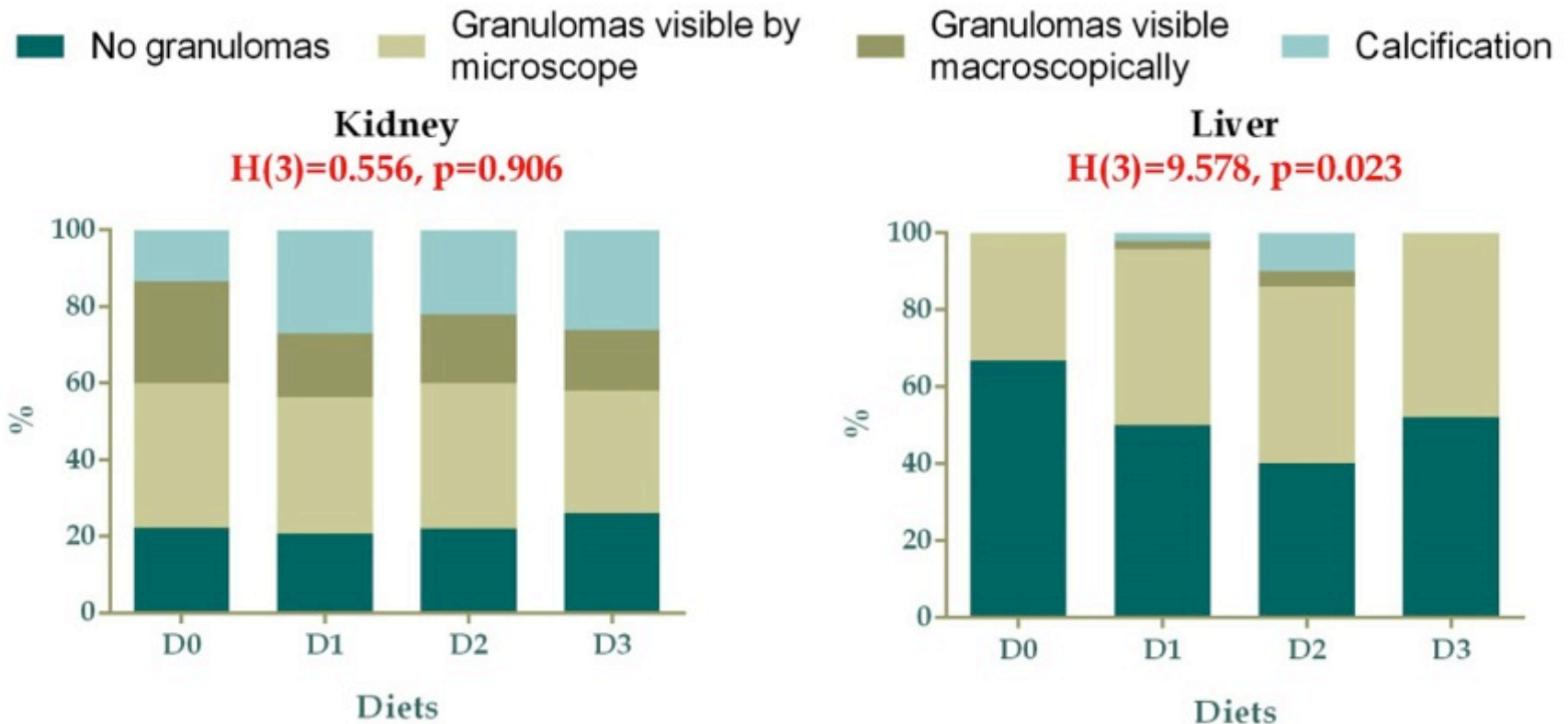
## *Evaluation of granulomas*

- The overall condition of the fish assessed as the sum of the tissue's score was not affected by Vitamin D<sub>3</sub> supplementation



# Evaluation of granulomas

- A statistical significant difference between the diets exists only for the liver



# The effect of Ca/P ratio

# *Experimental design*

- ✓ June 2015 – October 2015
- ✓ 9 diets

	<b>Diets</b>								
<b>gkg<sup>-1</sup></b>	1	2	3	4	5	6	7	8	9
<b>Ca</b>	5,49	6,44	12,83	5,49	10	20	5,55	15	30
<b>P</b>	6,44	6,44	6,42	10	10	10	15,58	15	15

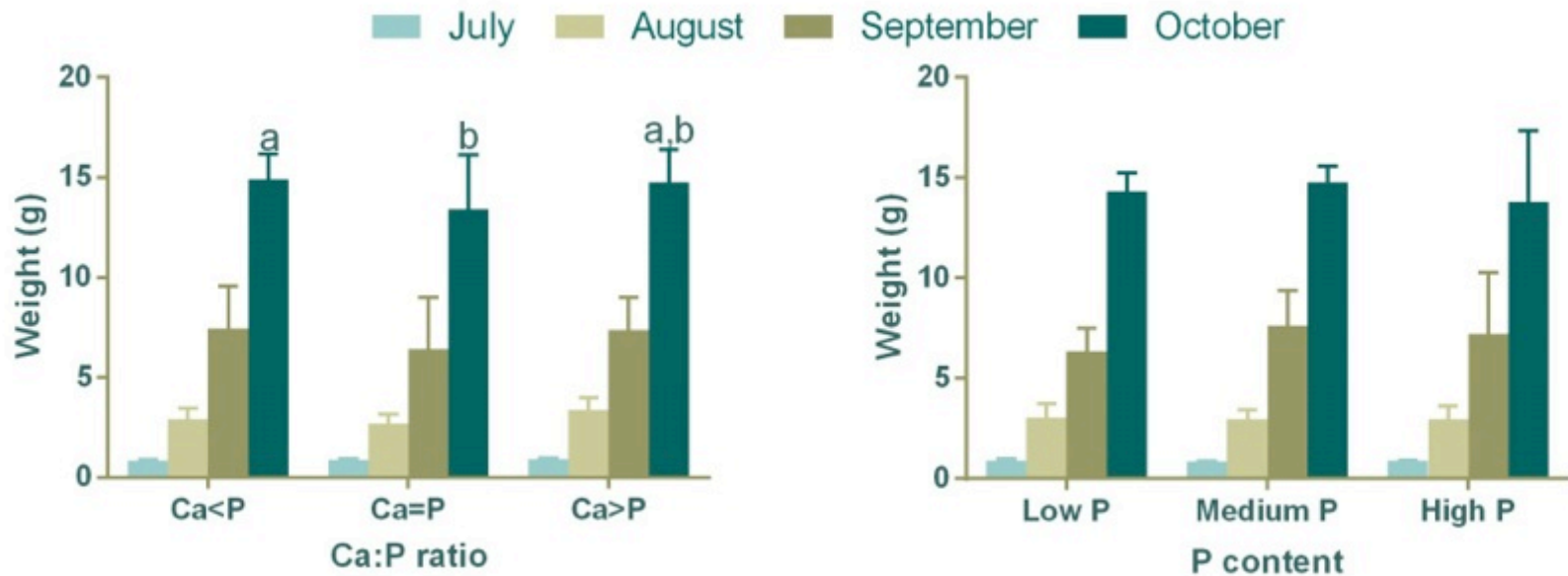
- ✓ 27 tanks (500 l)
- ✓ 1350 fish (Initial weight ~ 1g)

## *Experimental design*

- ✓ **Samplings every month**
  - Growth performance
  - Evaluation of granulomas
  - Histology
  - Plasma analysis

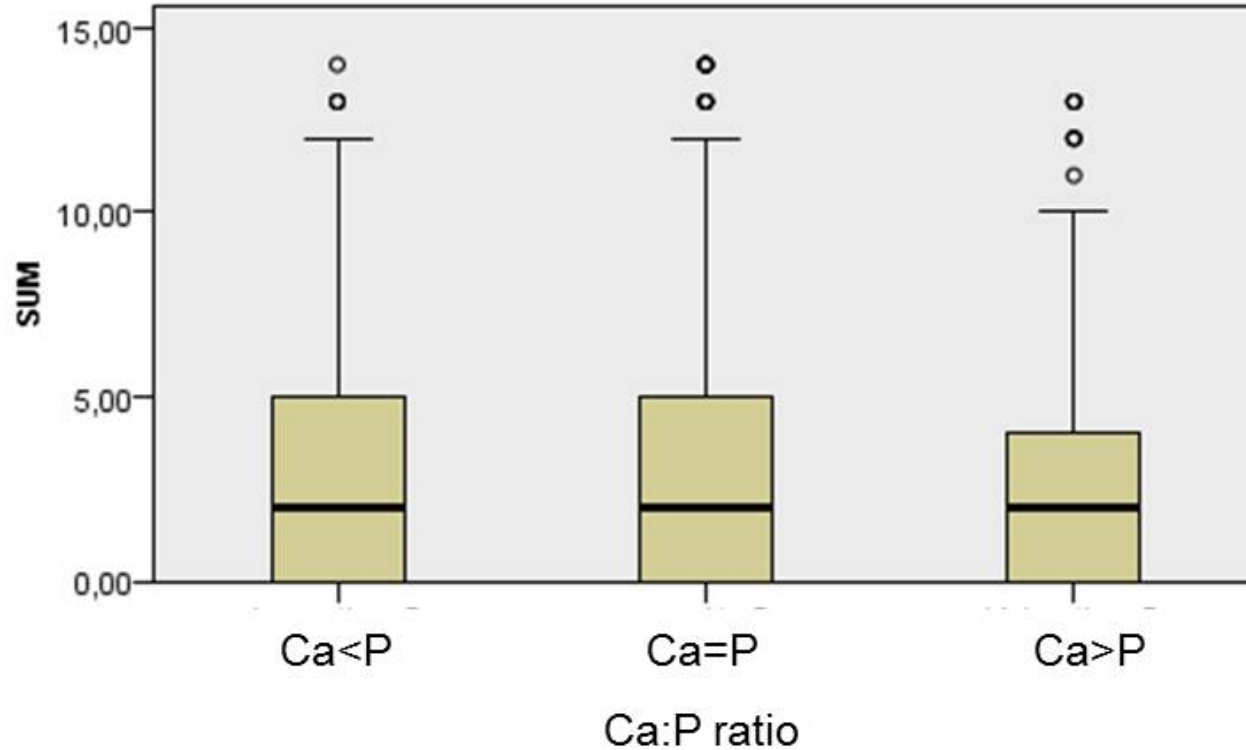
# Growth performance

- Juvenile meagre grew from ~1 g to ~15 g



# *Evaluation of granulomas*

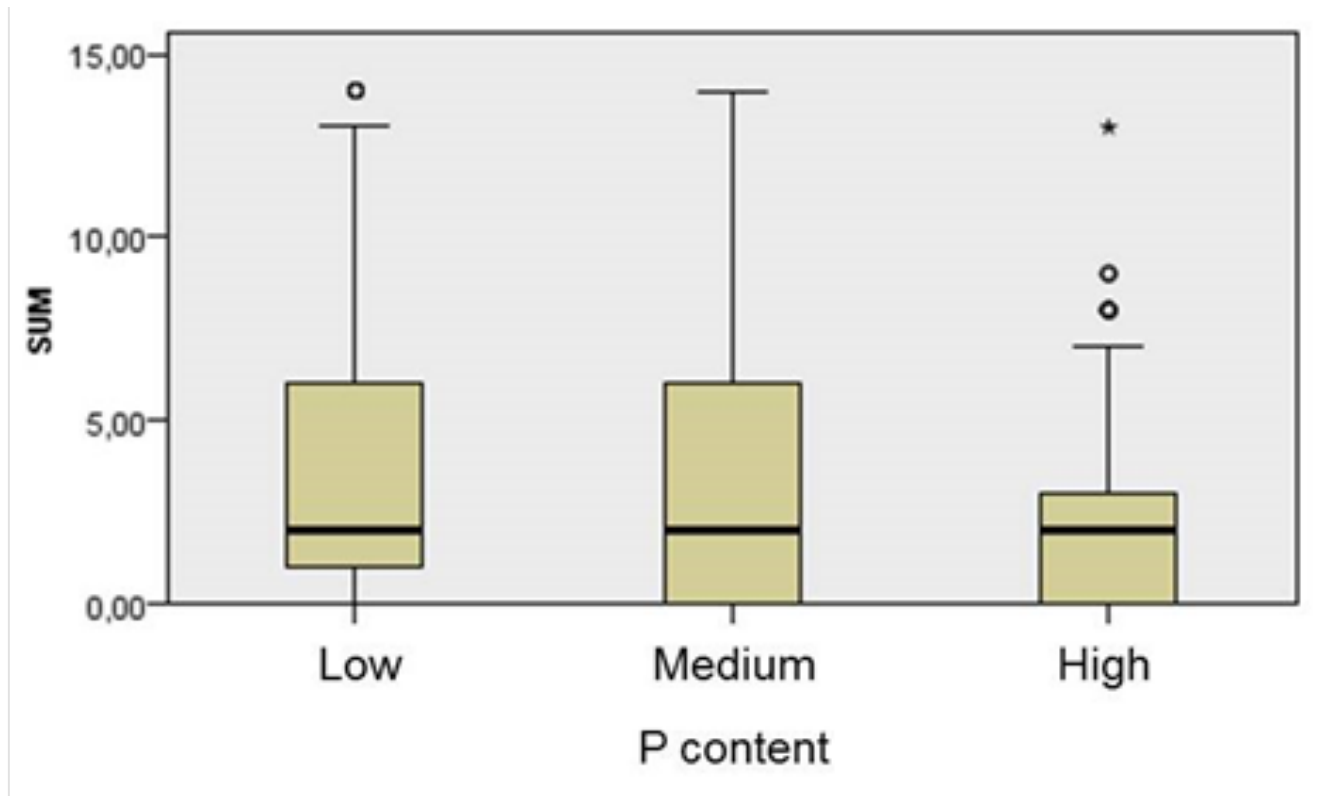
- The medians of the groups with different Ca:P ratio are similar





# *Evaluation of granulomas*

- The medians of the groups with **high** and **low** P content are significant different



The effect of high plant protein in  
combination with P inclusion

# *Experimental design*

August 2016 – November 2016

4 diets

	FM	PP + High P	PP + Medium P	PP
<b>Fishmeal (%)</b>	60,18	14,00	14,00	14,00
<b>Phosphate (g/kg)</b>	14,32	14,00	9,75	5,46

12 tanks (500 l)

600 fish (Initial weight ~ 2g)

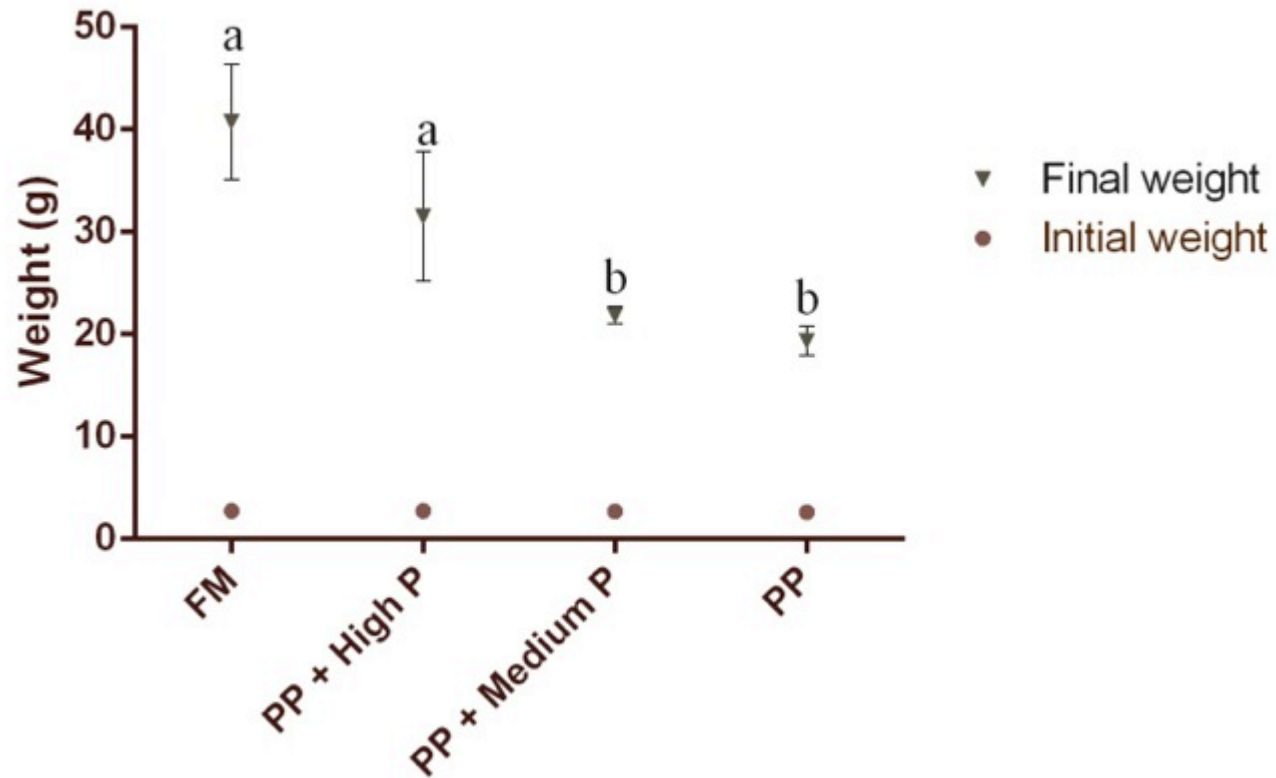
# *Experimental design*

## **Samplings every month**

- Growth performance
- Evaluation of granulomas
- **Histology**
- **Plasma analysis**

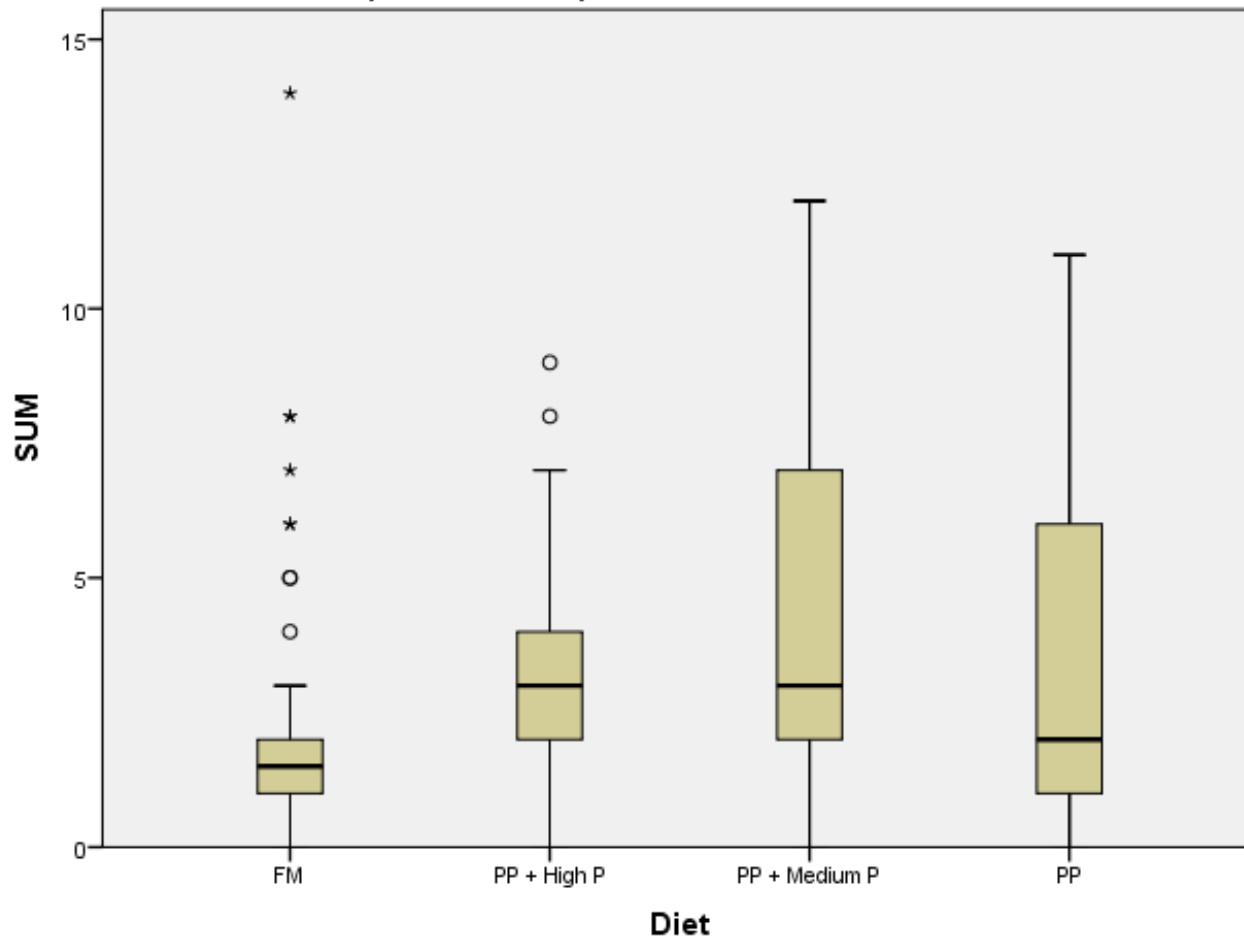
# Growth performance

- Juvenile meagre grew from ~2 g to ~40 g



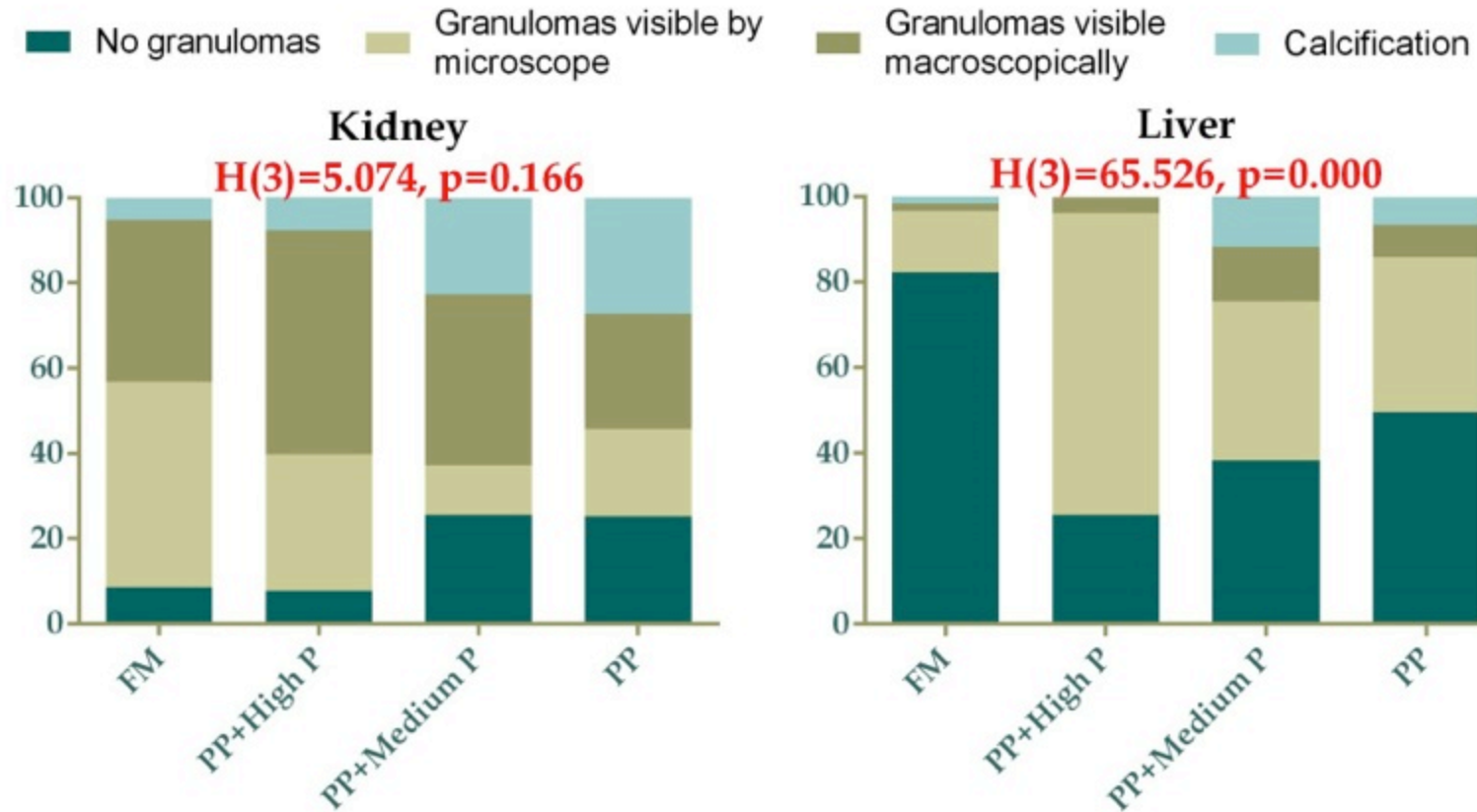
# *Evaluation of granulomas*

- FM median was significant different from PP+High P and PP +Medium P median



# Evaluation of granulomas

- A statistical significant difference exists only for the liver



# General conclusions

- Vitamin D supplementation did not affect the development of the disease
- High P in the diet seems to improve the condition (but not dramatically)
- Plant protein negatively affects the condition (the effect here is much higher)



# Hypothesis #2

## Infectious agent

Journal of Fish Diseases 2012

doi:10.1111/jfd.12015

### **Systemic nocardiosis in a Mediterranean population of cultured meagre, *Argyrosomus regius* Asso (Perciformes: Sciaenidae)**

A Elkesh<sup>1</sup>, K P L Kantham<sup>2</sup>, A P Shinn<sup>1</sup>, M Crumlish<sup>1</sup> and R H Richards<sup>1</sup>

<sup>1</sup> Institute of Aquaculture, University of Stirling, Stirling, UK

<sup>2</sup> Nireus S A, Hiliadou Doridos, Focida, Greece

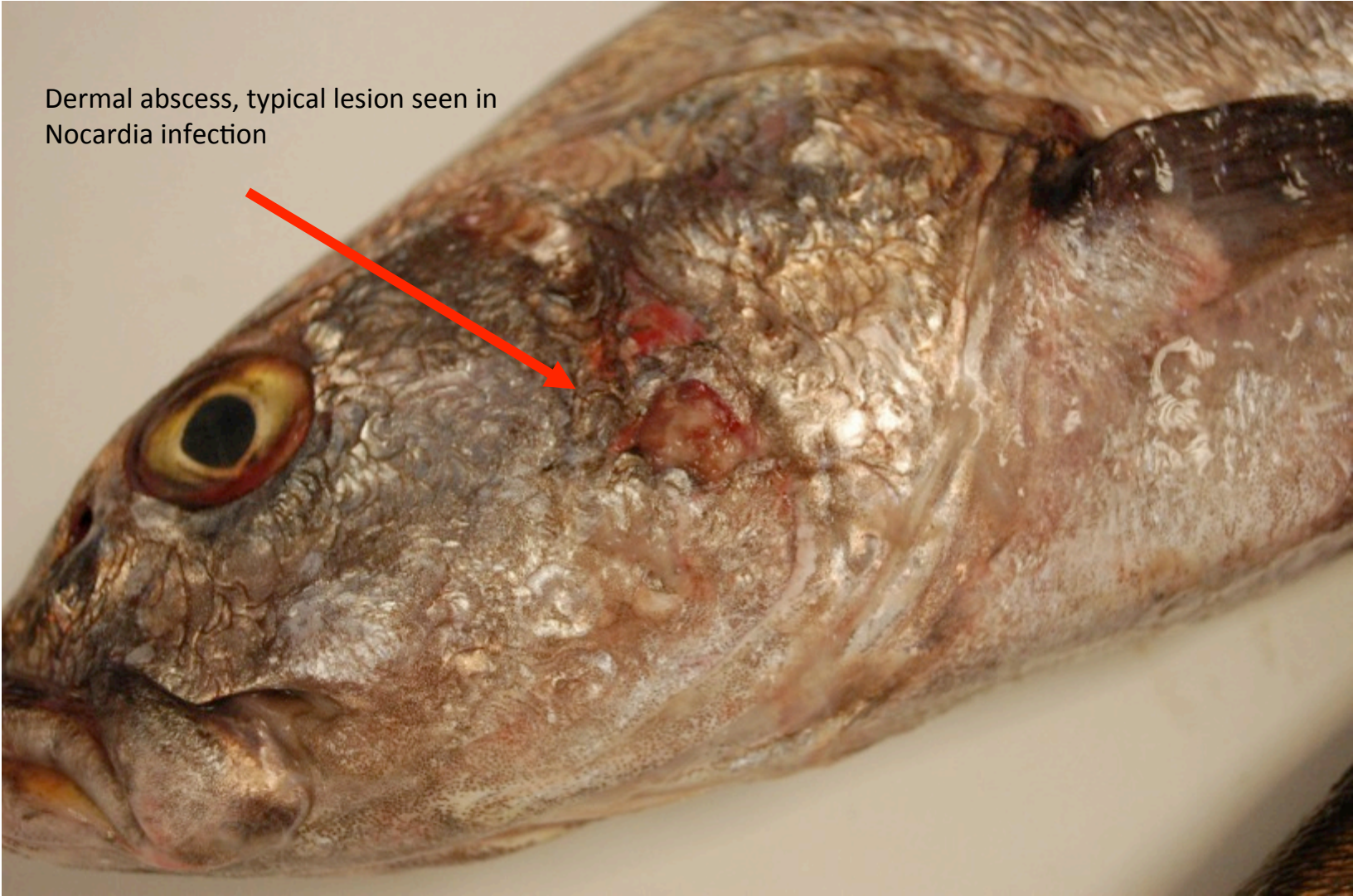


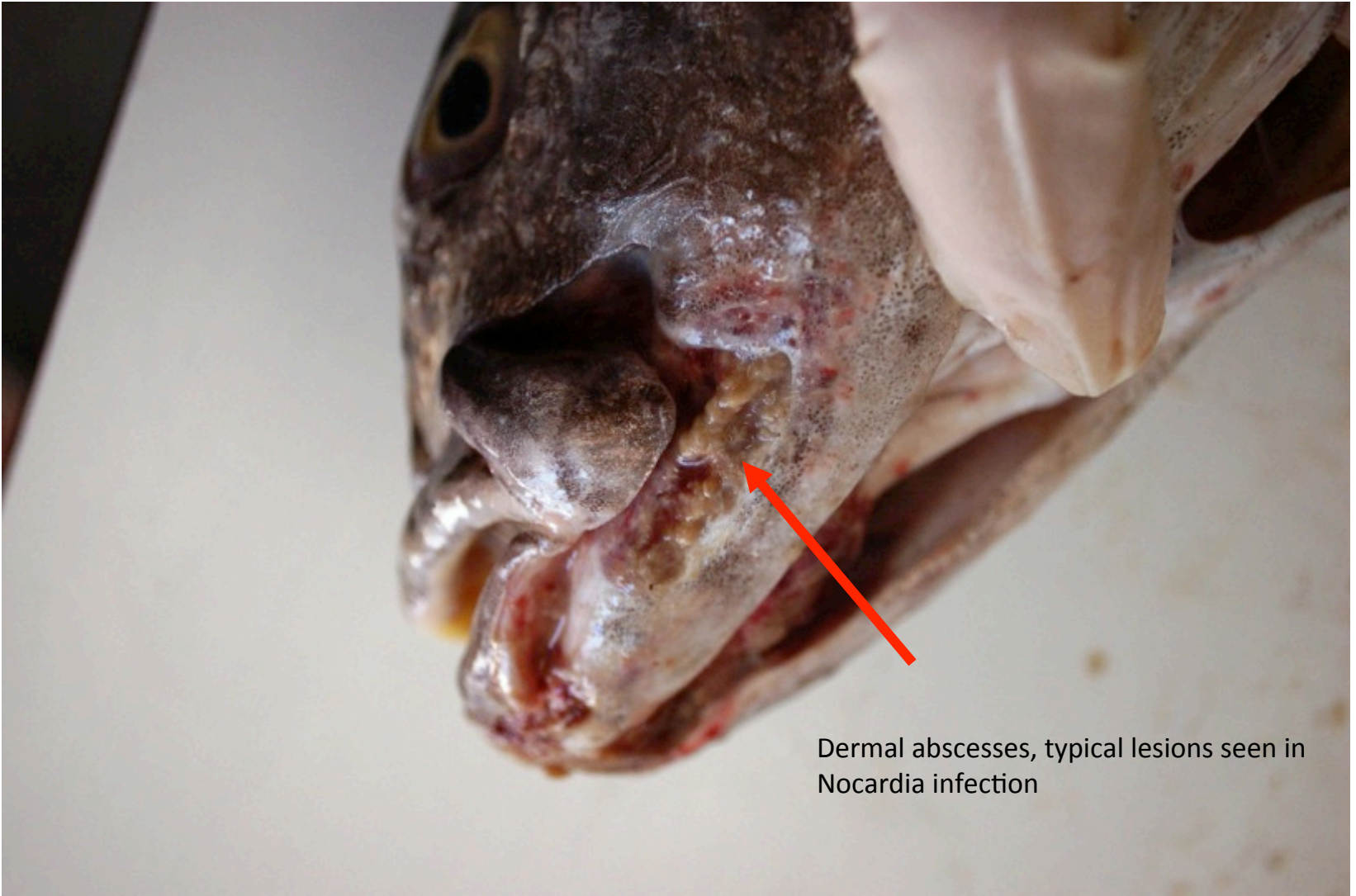
After 3 years we finally have it!!!



Severe ulceration of the skin

Dermal abscess, typical lesion seen in  
Nocardia infection





Dermal abscesses, typical lesions seen in Nocardia infection

# BLAST analysis showed a 100% identity with *Nocardia seriolae*

https://blast.ncbi.nlm.nih.gov/Blast.cgi#91204703

NIH U.S. National Library of Medicine NCBI National Center for Biotechnology Information katharios My NCBI Sign Out

**BLAST** » **blastn suite** » RID-32PGBJTJ014 Home Recent Results Saved Strategies Help

### BLAST Results

Your search is limited to records matching entrez query: txid37332 [ORGN].  
[Edit and Resubmit](#) [Save Search Strategies](#) [Formatting options](#) [Download](#) [YouTube](#) [How to read this page](#) [Blast report description](#)

#### Nucleotide Sequence (580 letters)

RID [32PGBJTJ014](#) (Expires on 11-21 15:08 pm)

**Query ID** lc|Query\_188757  
**Description** None  
**Molecule type** nucleic acid  
**Query Length** 580

**Database Name** nr  
**Description** Nucleotide collection (nt)  
**Program** BLASTN 2.5.1+ [Citation](#)

Other reports: [Search Summary](#) [Taxonomy reports](#) [Distance tree of results](#)

[+ Graphic Summary](#)  
[- Descriptions](#)

#### Sequences producing significant alignments:

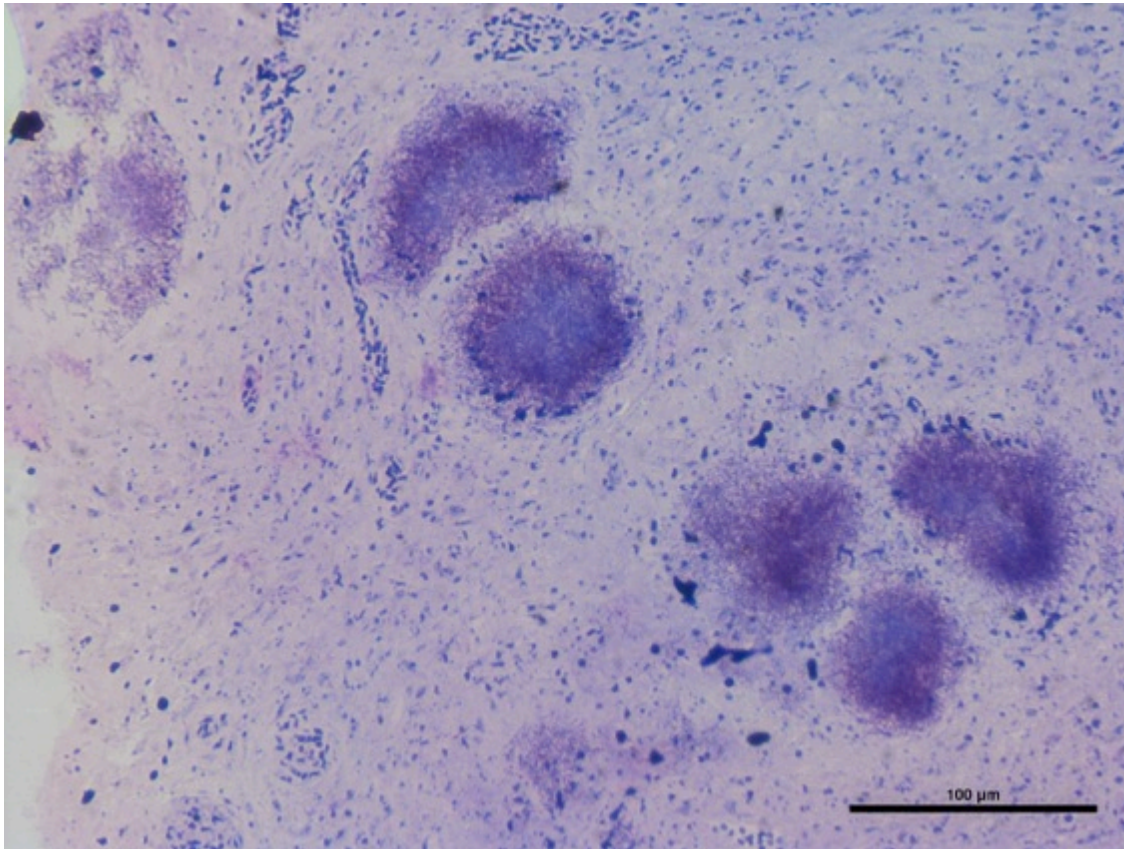
Select: [All](#) [None](#) Selected:0

[Alignments](#) [Download](#) [GenBank](#) [Graphics](#) [Distance tree of results](#)

	Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/>	<a href="#">Nocardia seriolae strain LH1347 16S ribosomal RNA gene, partial sequence</a>	1072	1072	100%	0.0	100%	<a href="#">KY029027.1</a>
<input type="checkbox"/>	<a href="#">Nocardia seriolae partial 16S rRNA gene, isolate AT3HP14</a>	1072	1072	100%	0.0	100%	<a href="#">LT221194.1</a>
<input type="checkbox"/>	<a href="#">Nocardia seriolae partial 16S rRNA gene, isolate HL4RS10</a>	1072	1072	100%	0.0	100%	<a href="#">LT221160.1</a>
<input type="checkbox"/>	<a href="#">Nocardia seriolae strain WEL-NS01 16S ribosomal RNA gene, partial sequence</a>	1072	1072	100%	0.0	100%	<a href="#">KM387284.1</a>

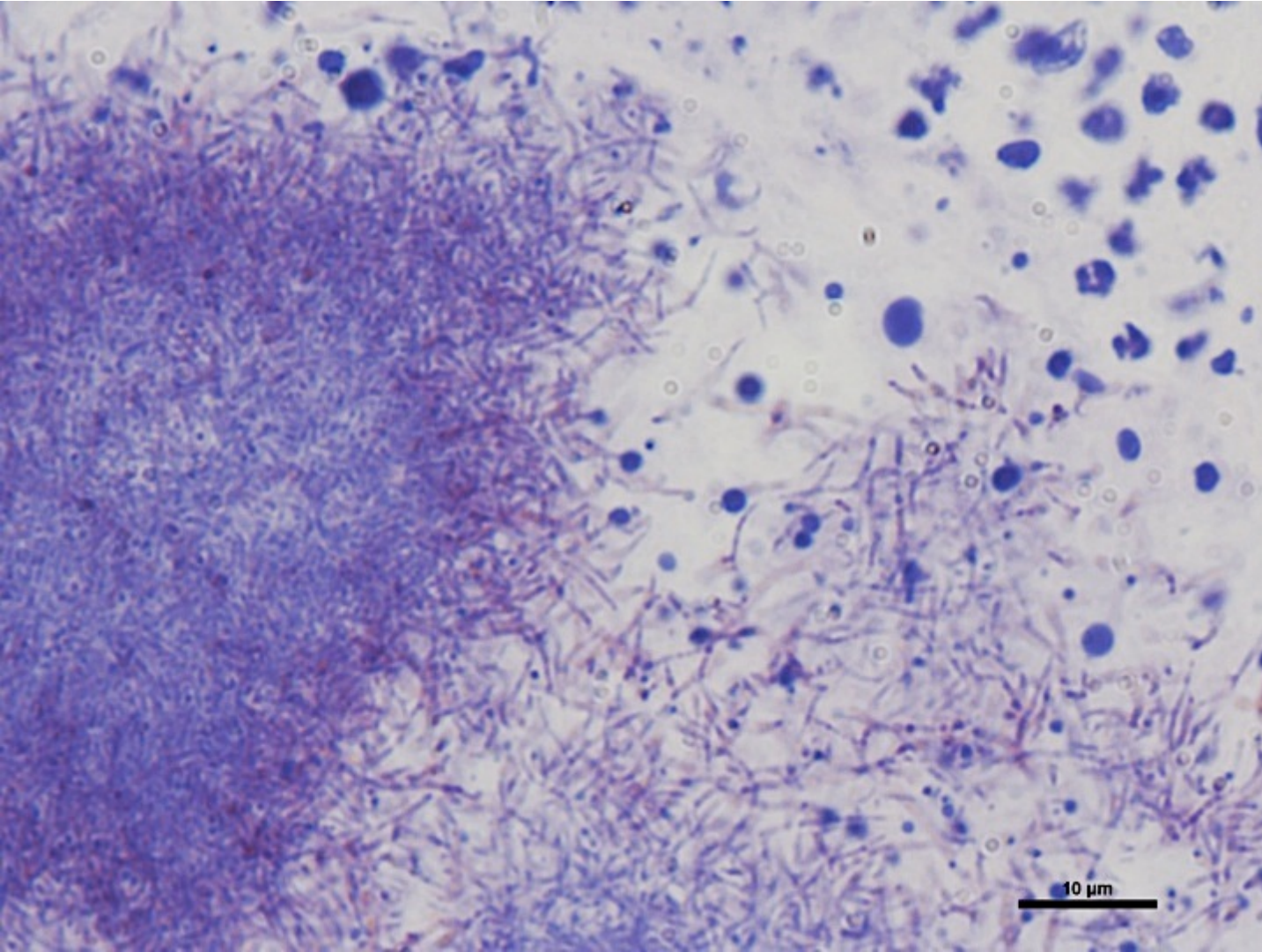


# Histopathology



Section of dermal lesion showing bacterial colonies.  
Ziehl Neelsen stain





Higher magnification of the previous picture, showing acid-fast elongated and branching bacteria, consistent with the typical morphology of *Nocardia* spp.  
Ziehl Neelsen stain

# Further hypothesis for granulomatosis

- Other nutritional metabolic factors
- Genetic background (diversity of broodstock)
- Unknown and invisible pathogen



- D24.1 submitted
- D24.2 submitted
- D24.4 submitted
  
- D24.5 (to be submitted very soon)
- D24.14 (at the end of the project)