

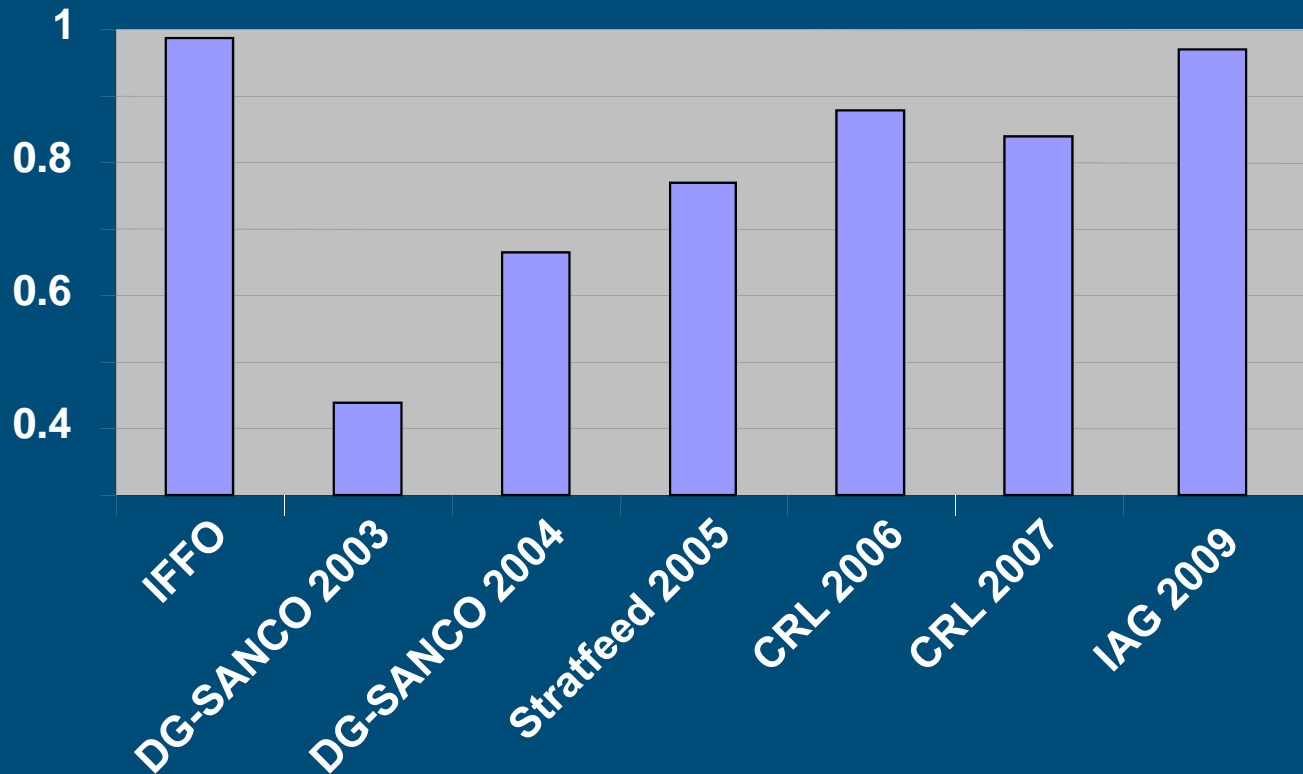
# New developments in classical microscopy; what can be expected for the official control?

L.W.D. van Raamsdonk



# Classical microscopy in official control

❖ Ringtests: historical overview (0.1 % MBM; 5 % fish)



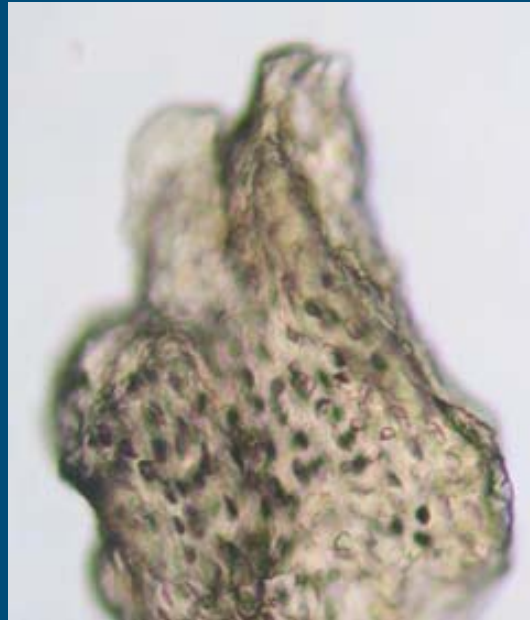
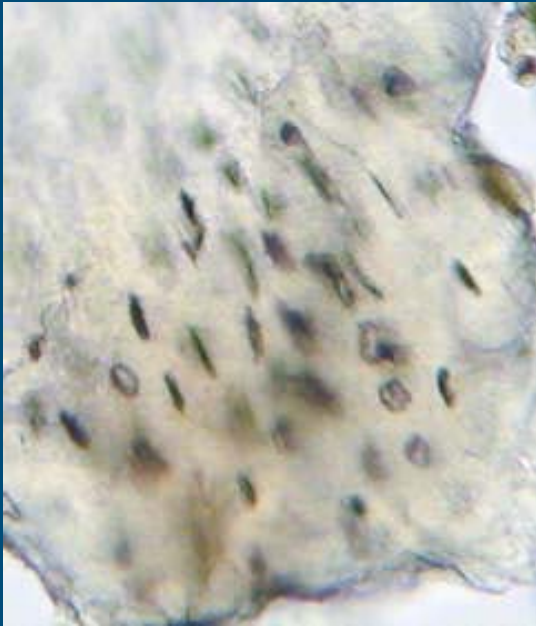
# Classical microscopy in official control

## ■ Topics

- ⇒ Morphological analysis of bone fragments
- ⇒ Modified sedimentation procedure
- ⇒ Combination method: *in situ* immunochemistry

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## ❖ Morphology of bone fragments



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## ❖ Multivariate analysis of bone fragments

⇒ 56 characteristics of 1143 lacunae from 25 samples

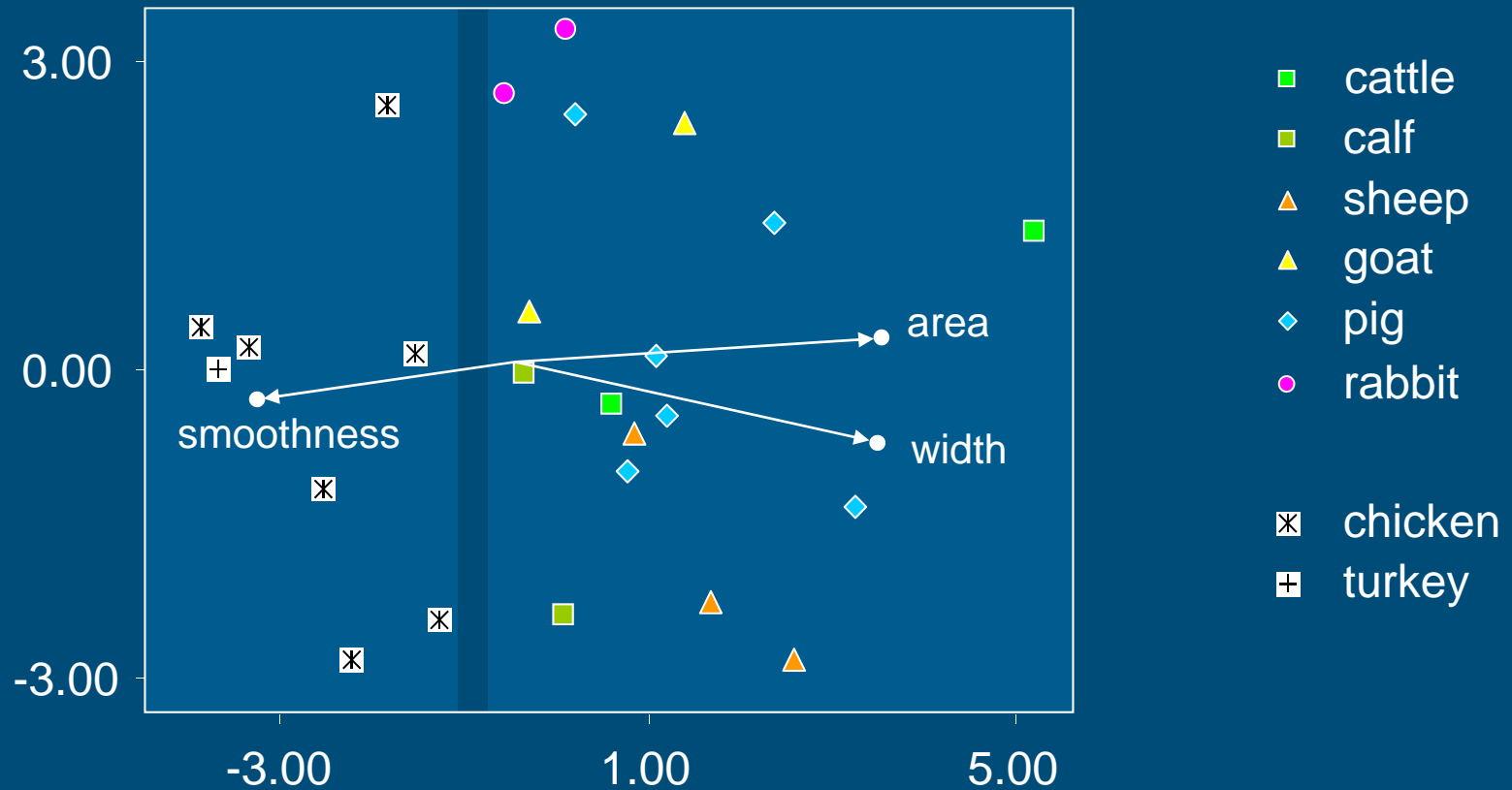
⇒ Selection of 10 characters (limitation of redundancy)

⇒ Averages for 25 samples

⇒ Principal component analysis

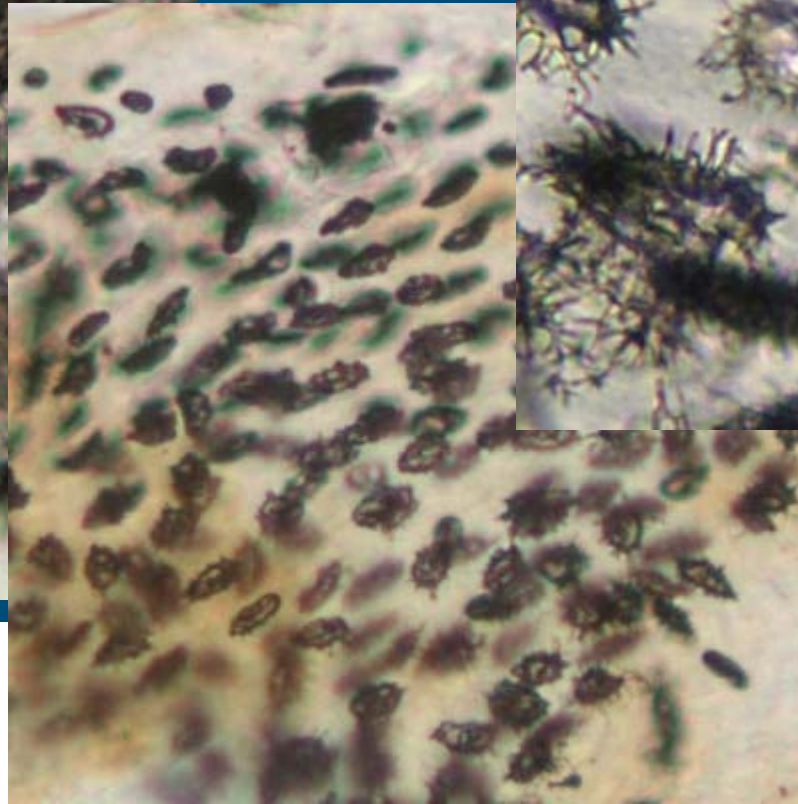
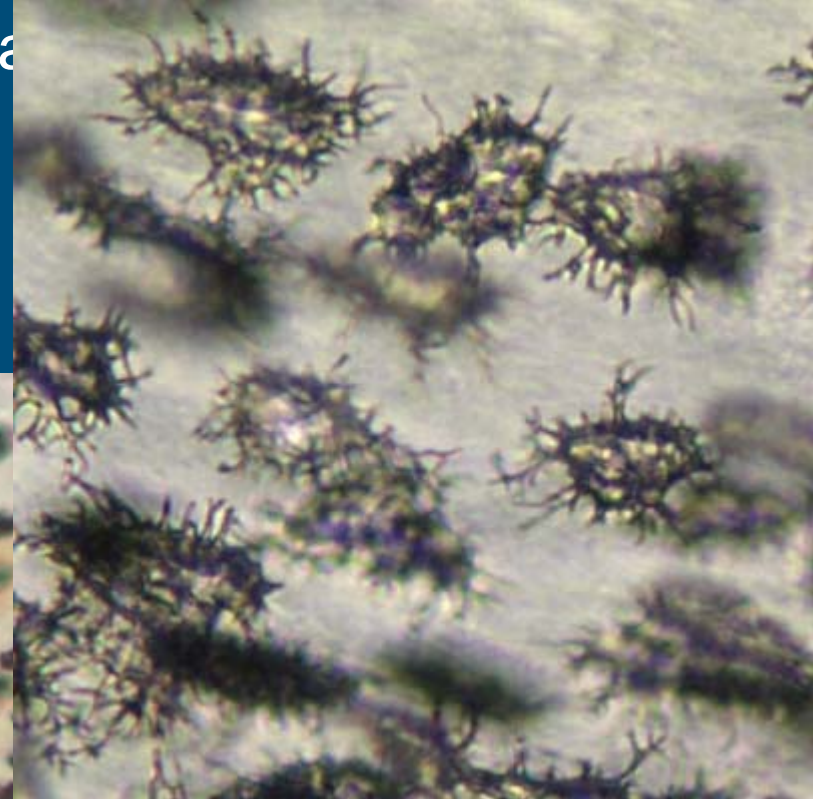
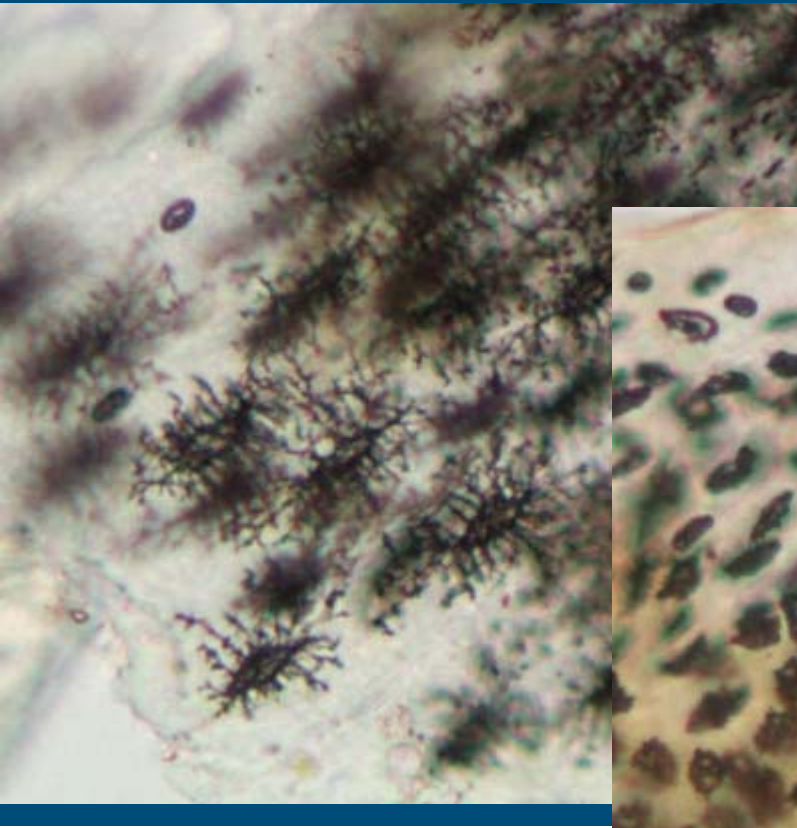
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## ❖ Multivariate analysis of lacuna morphology



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## ❖ Analysis of density and canalicula



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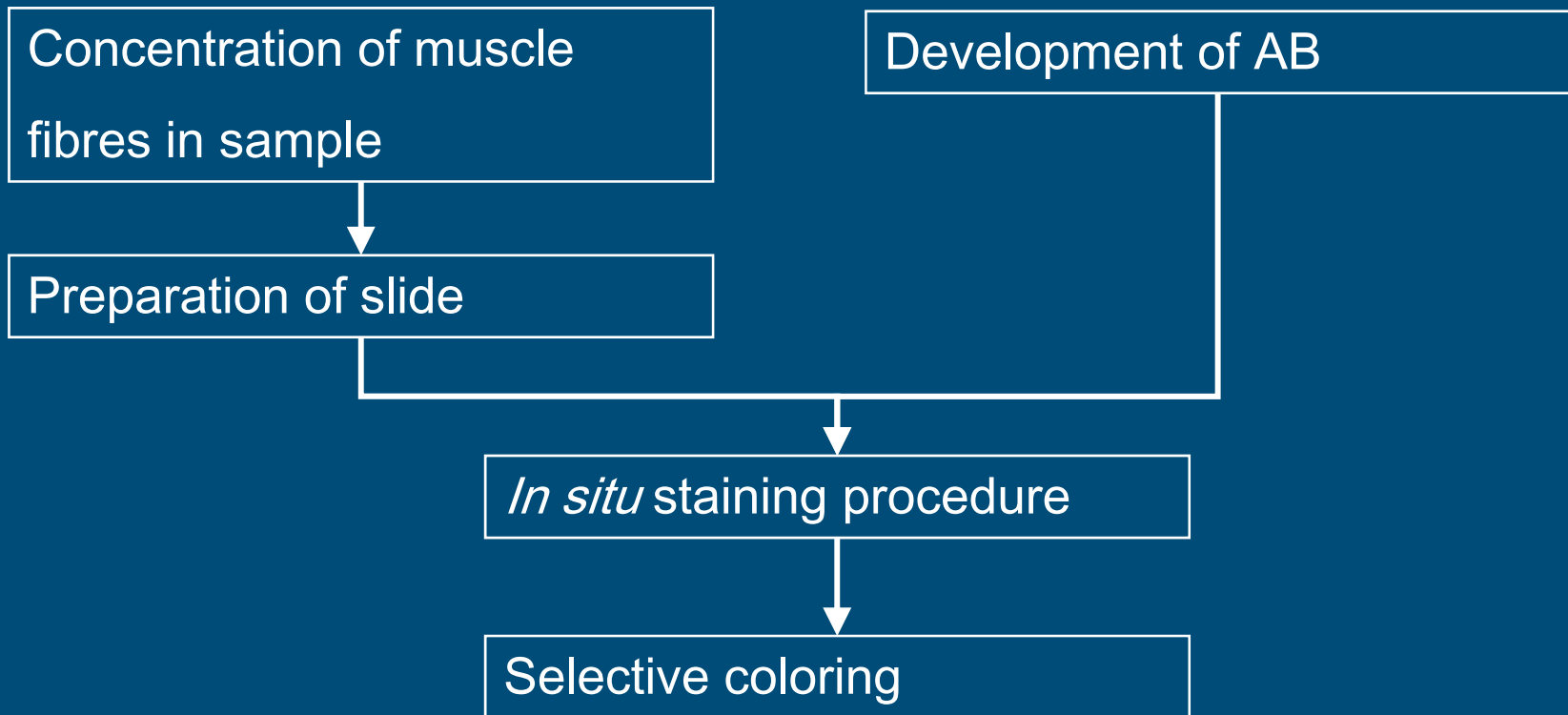
## ❖ *In situ* hybridisation prerequisites

⇒ Certain samples with only muscle fibres

⇒ Troponin I ABs in development

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## ❖ *In situ* hybridisation



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## ❖ Modified sedimentation procedure (MBM : feed = 50/50)

TCE/heptane	muscle recovery	flotation	concentration
70/30	55 %	2.7 %	20.37
75/25	80 %	7.1 %	11.27
80/20	75 %	17.4 %	4.3
90/10	97 %	54.6 %	1.78

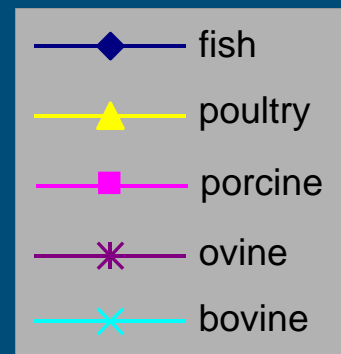
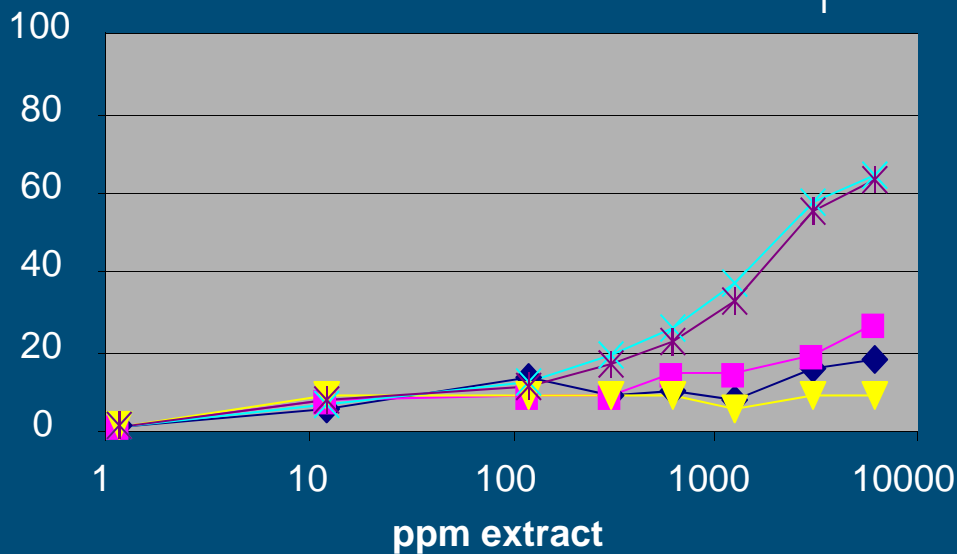
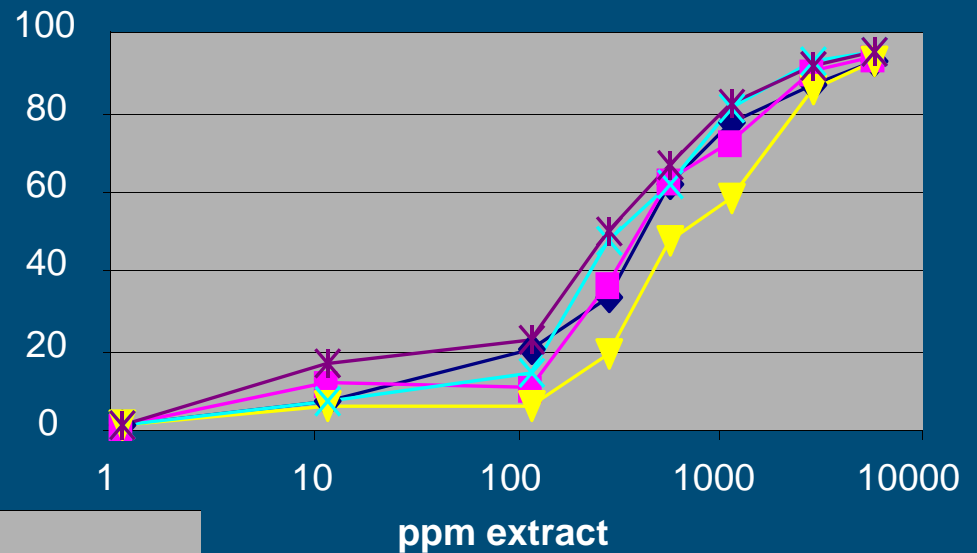
Feed with 0.1 % MBM (f=0.5)

TCE	bone fragment rec.	sediment	concentration
100	100 %	2.5 %	40.0

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## ❖ Immunochemistry

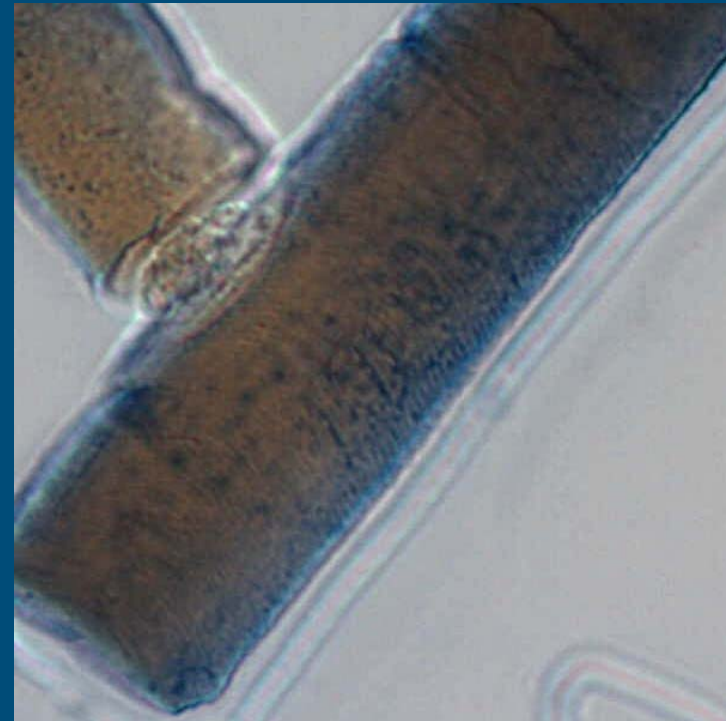
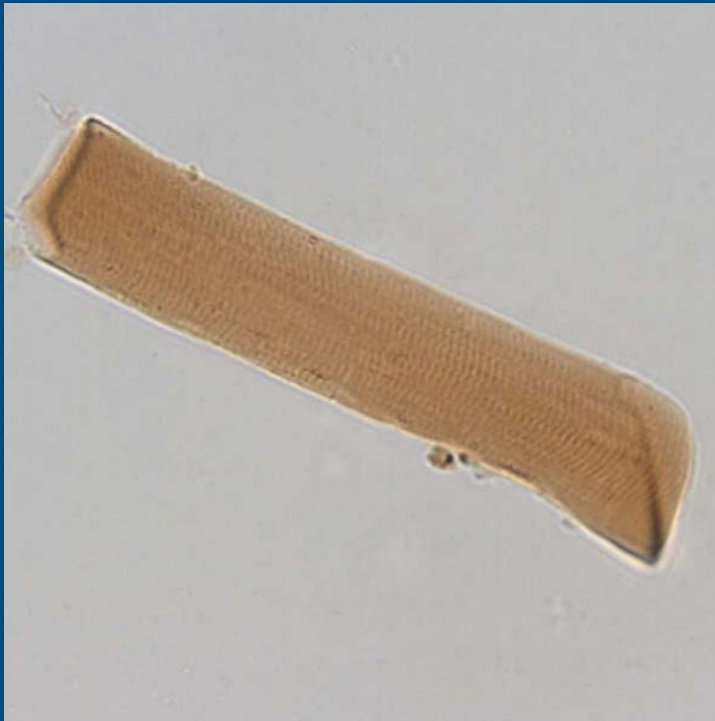
- General antibodies
- Target: ruminants troponin I



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## ❖ *In situ* hybridisation 1

- Specific reaction of AB with troponin I in sarcomeres bovine muscle fibres



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## ❖ *In situ* hybridisation 2

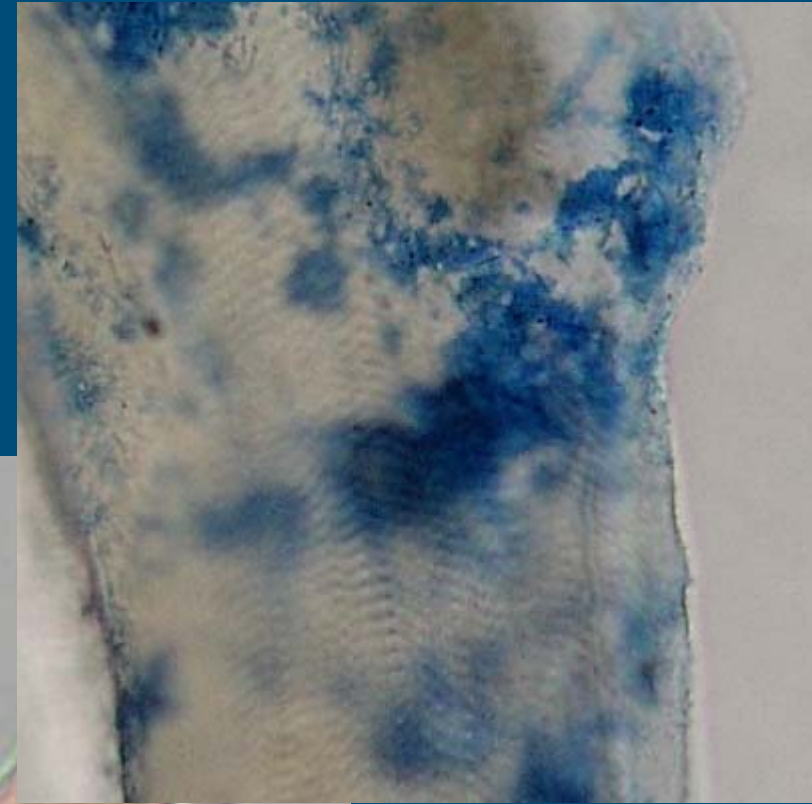
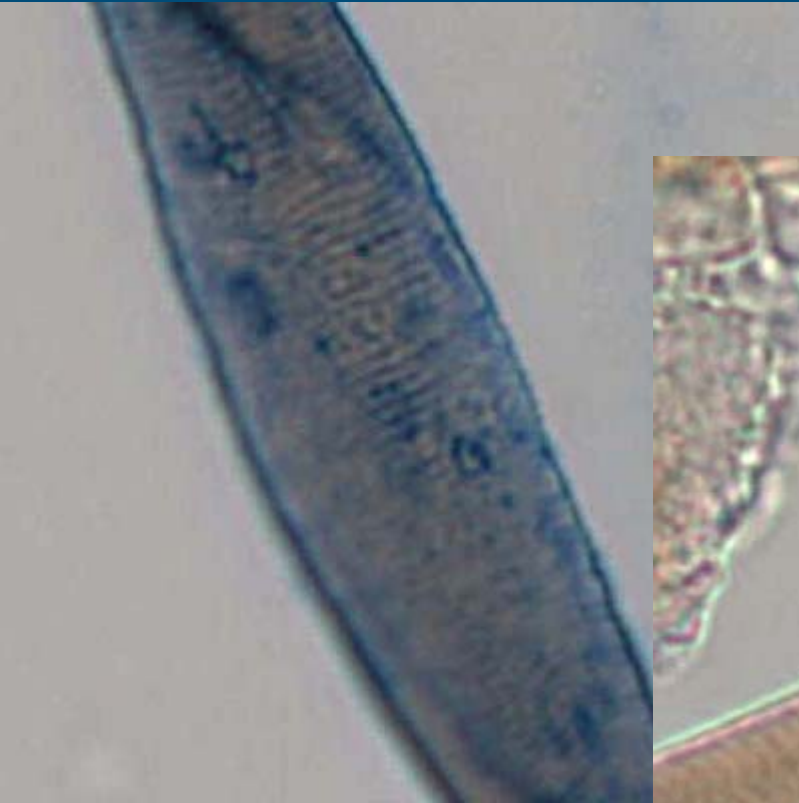
- Aspecific reaction of AB with membrane proteins or fosfolipids in fish muscle fibres



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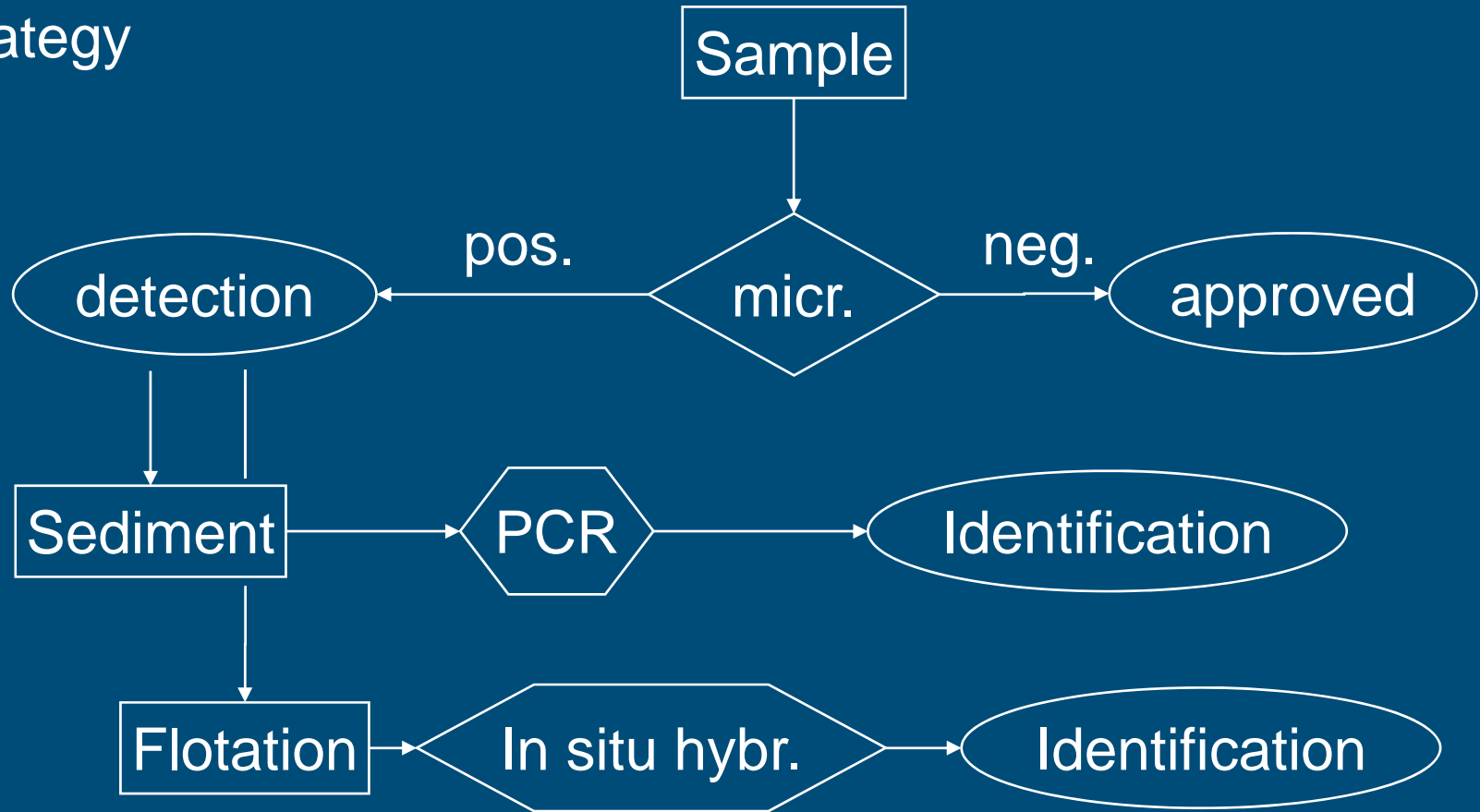
## ❖ *In situ* hybridisation 3

- Close ups of sarcomere response



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## ❖ Strategy



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## ❖ Prospects

- ⇒ Morphologic/density features in bone fragments
- ⇒ Immunochemical identification of muscle fibres
- ⇒ Testing and approval procedure for ABs

# Classical microscopy in official control

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# Thank you for your attention

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