

Rapid quantification and classification of *Nosema* spp. spores and *M. mellificae* cysts using automated microscopy and artificial intelligence

Iratxe Perales¹, Naoufal Amrani Ouazzani¹, María Benito², Eduardo José García-Vicente², Ana Pérez³, María Martín Domínguez⁴, Noelia Hermosilla Plaza², Juan Manuel Alonso⁴, David Risco³

¹Microfy Systems S.L, Av. Carrilet 243, 1º 2º 08907 Barcelona, Spain

²Neobéitar S.L, Av. Alemania 6, 1 B, 1001 Cáceres, Spain

³Department of Animal Medicine, Faculty of Veterinary Medicine, University of Extremadura, 10003 Cáceres, Spain.

⁴Department of Animal Health, Infectious Diseases Unit, Faculty of Veterinary Medicine, University of Extremadura, 10003 Cáceres, Spain.

The global decline in honeybee colonies in recent decades is generating a significant crisis in the sector. This is due to multiple factors, although pathogens are considered the major cause.

Nosemosis, caused by *Nosema apis* (type A) and *Nosema ceranae* (type C), is a key factor in colony loss, as a high rate of infection by *Nosema* spp. significantly weakens colonies, and exposes them to higher mortality when combined with other additional stressors. Nosemosis infection rate has been proven to be a useful parameter to monitor actual health of bee colonies.

The absence of clear clinical signs caused by *Nosema* type C makes diagnosis very difficult. Therefore, laboratories and veterinary technicians rely on standardized methods on honeybee samples to confirm and quantify the presence of *Nosema* spp.. However, methods used are PCR and/or optical microscopy, which both require expert performance and are labor and/or resource intensive.

This abstract presents a solution for autonomous full-scanning of samples with an affordable automated brightfield microscope, and subsequent detection/counting of *Nosema* spp. spores, with high discrimination accuracy among the other elements that appear, and species classification. Additionally, amoebiasis (*Malpighamoeba mellificae*) is also detected through cysts presence, which is commonly found in collaboration with nosemosis.

The device autonomously scans 20 fields (x600) of a volumetric chamber and processes the images in the cloud for rapid quantification, in 7 minutes/sample (including sedimentation).

The training dataset has been developed by Microfy, with samples provided by the Spanish Central Veterinary Laboratory. The AI pipeline developed includes: 1) autofocus, 2) MaskR-CNN detection, 3) (U-Net + OpenCV) to quantify width and length required to classify *N. apis*/*N. ceranae*, and 4) MLP, multilayer perceptron, based on color/refraction/geometry.

The achieved F1-score accuracy was 95.25% on validation dataset. The experimental validation (n = 50) was carried out by Neobéitar SL and the University of Extremadura. The validation concluded with a higher sensitivity than in the Neubauer chamber, and a high correlation with manual counting in an optical microscope. (R2 =0.94, p<0.05, specificity >80%), which confirms the automated device as a useful, affordable and easy-to-use support tool for diagnosis, and recurrent monitoring of nosemosis/amoebiasis.

A composite image featuring a grayscale photograph of a hand adjusting a microscope's objective lens. The background is white with a faint, blue, geometric network pattern of interconnected lines and polygons. The text is overlaid on the right side of the image.

Microfy.AI

Affordable, simple and fast quantification and classification of Nosema spp. spores and M. mellificae cysts using automated microscopy and artificial intelligence



IRATXE PERALES
CEO at MICROFY SYSTEMS



Microfy Systems. Who we are



Iratxe Perales

CEO and co-founder



Eduard López

Co-founder and
Mechatronics Design



Naoufal Amrani

Artificial Intelligence
Lead



Àlvaro Suarez

Fullstack Dev. Engineer



Asma Ghorab

PhD. Melissopalinologist
AI models trainer



Julian Rodriguez

Community manager
and customer service



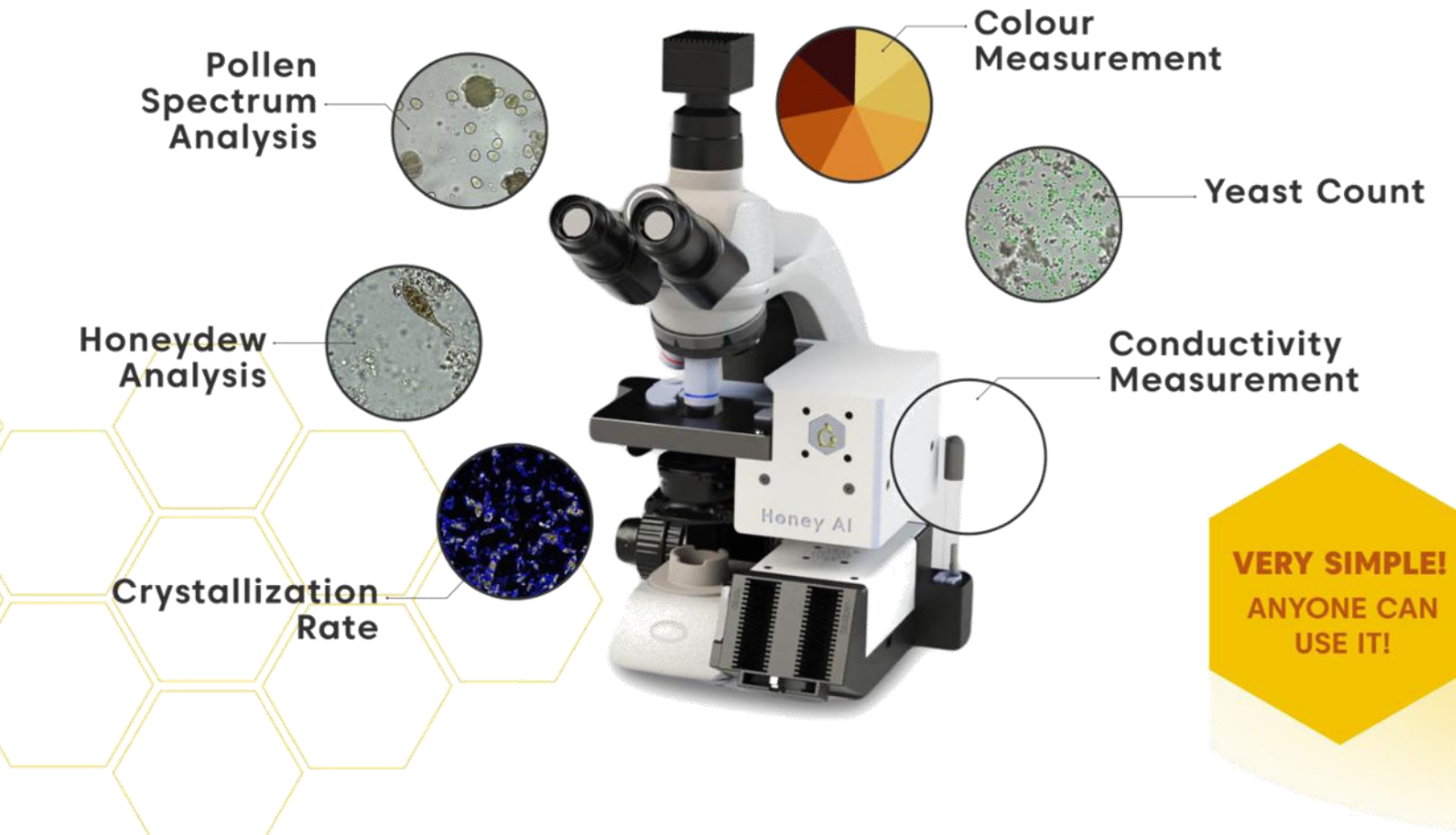
Miguel Moreno

Firmware Engineer





HoneyAI. What is it?



www.honey-ai.com

AFFORDABLE

**AUTOMATED &
EASY-TO-USE**

VERSATILE

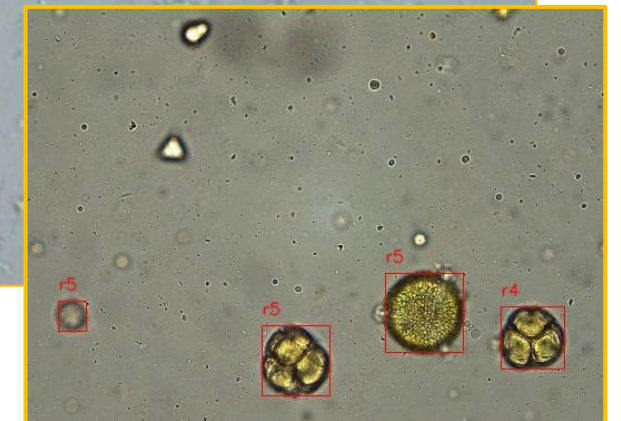
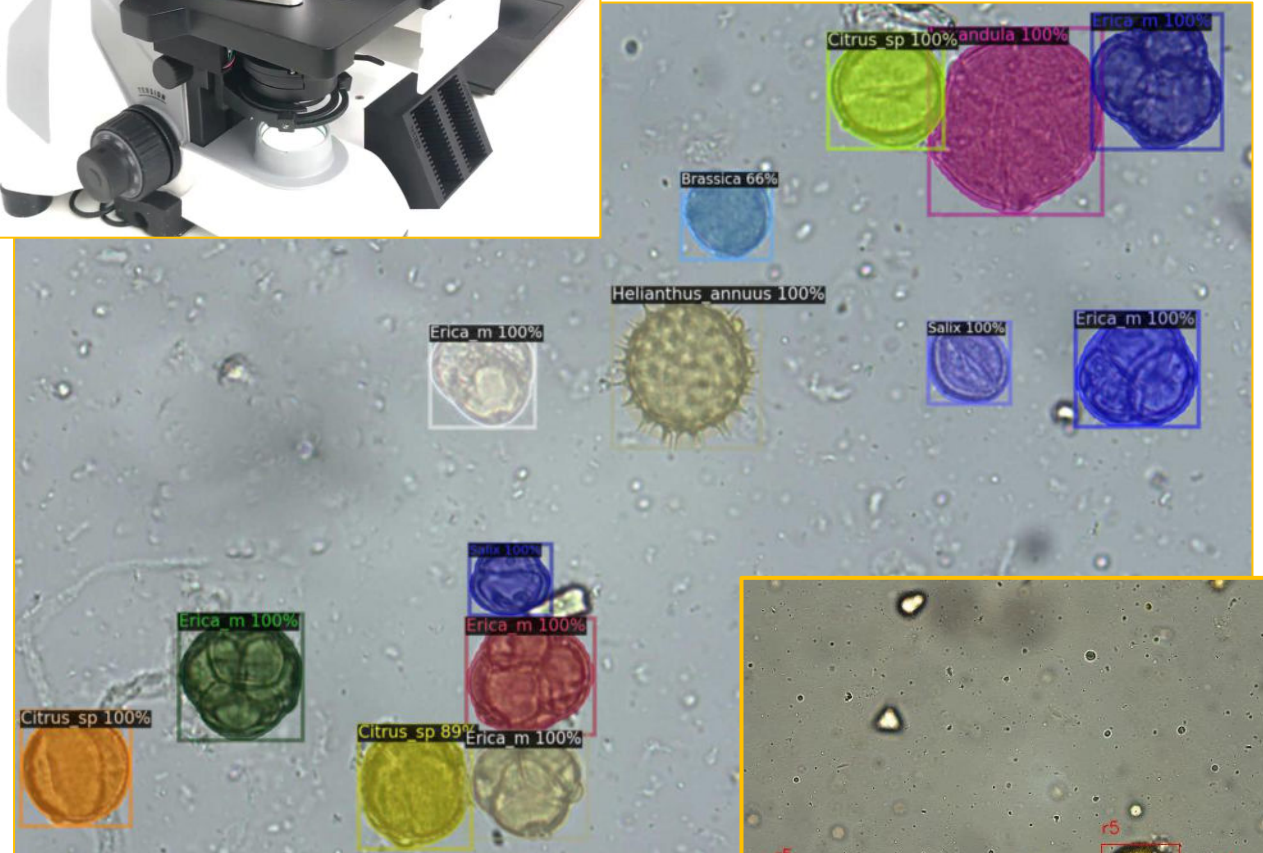
FAST

**VERY SIMPLE!
ANYONE CAN
USE IT!**



HoneyAI. What is it?

- Full Pollen Spectrum (honey & pellets)
- Yeasts concentration
- Starch detection
- Honeydew elements



Nosem AI

AUTOMATED NOSEMA SPORES COUNTING





Nosemosis. Negative Impact on beekeeping

Nosemosis is a disease that affects bees, caused by the *Nosema* microorganism, within the group of fungi.

It parasitizes the cells of the inner wall of the bees' intestine, responsible for the digestion and absorption of nutrients, so **its damage negatively impacts to the bees on assimilating the food ingested.**

This usually results in malnutrition, weakness, and eventually, if combined with other external stress factors, can lead to their death.

Nosemosis infection rate has been proven to be a **useful parameter to monitor actual health of bee colonies.**



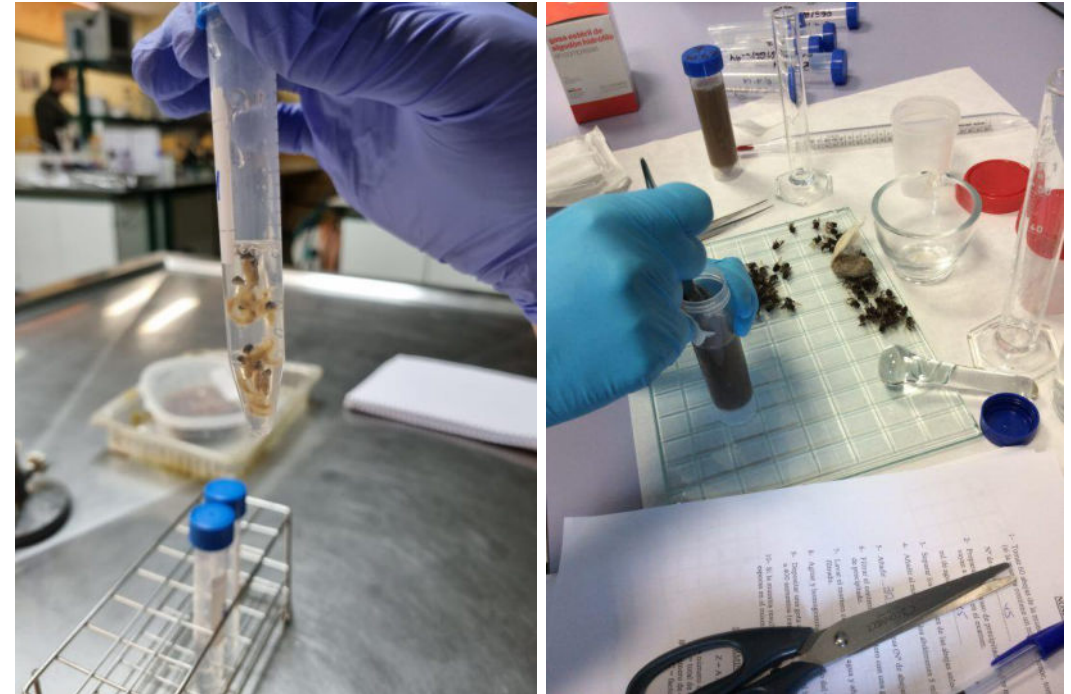


Nosemosis. How to diagnose?

The absence of clear clinical signs caused by *Nosema Ceranae* (type C) makes diagnosis difficult.

Therefore, laboratories and veterinary technicians rely on standardized methods on honeybee samples to confirm and quantify the presence of *Nosema* spp.

However, methods used are PCR and/or traditional optical microscopy, which both require expert performance and are labor and/or resource intensive





Nosemosis. Why is important to monitor?

There are effective treatments for Nosemosis, as the antibiotic Fumagilina, but the European Medicines Agency (EMA) banned its use in 2005 due to the risk of residues's accumulation in honey and bee wax.

So, what can be done by the beekeeper?

Easy answer: invest in early and preventive diagnosis.

So we can act before it's too late, mostly considering extra nutrients, food supplements and health good practices.





NosemAI. The development Project



Micrify.AI



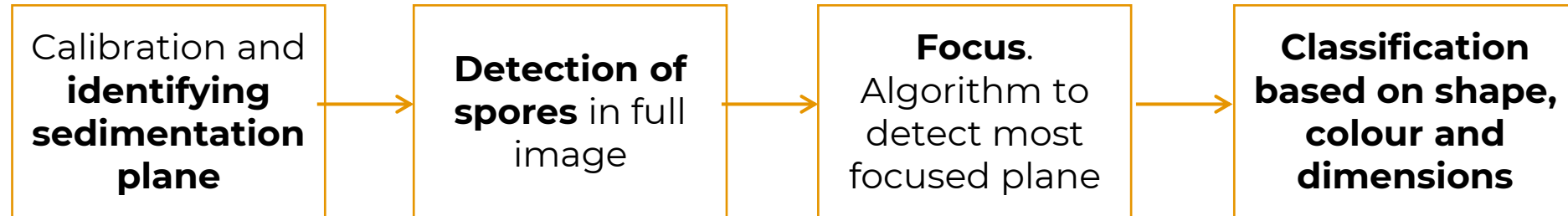
**Provisioning of samples
(Apis + Ceranae)**

- Development of datasets
- AI-algorithms
- Mechatronics

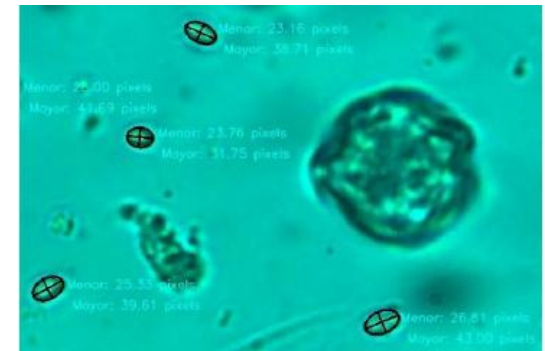
**Validation with
real samples**



NosemAI. The AI-module



1. Different detectors (Neural Networks) trained and tested, with Pytorch library.
2. Mask-RCNN selected – Data Augmentation predefined for the model – 20 epochs
3. 80% training – 20% validation
4. Different focus algorithms studied → *Sobel*/Algorithm selected – FOR EACH SPORE
5. Classification U-NET + OpenCV
 - RGB measuring (32 bins per canal)
 - Dimensions measuring (width and length)





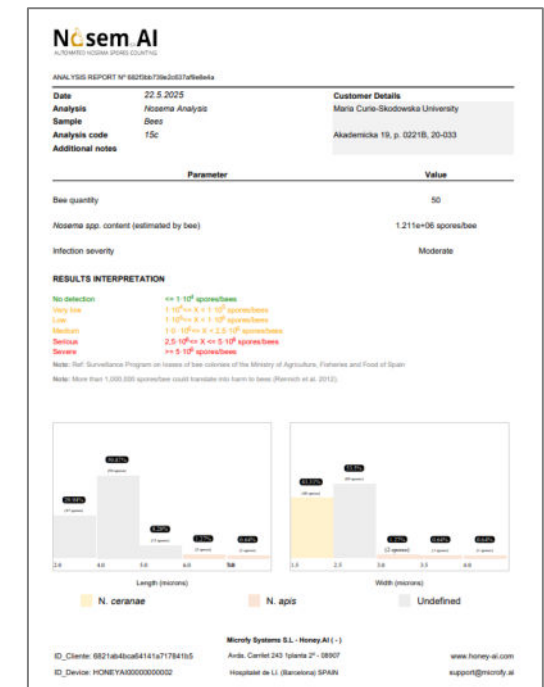
```
graph LR; A[Sample preparation  
(>30 adult bees)] --> B[Sample placement and  
launch of test]; B --> C[Sample scanning and  
cloud upload  
and process]; C --> D[PDF  
Generation for  
results];
```

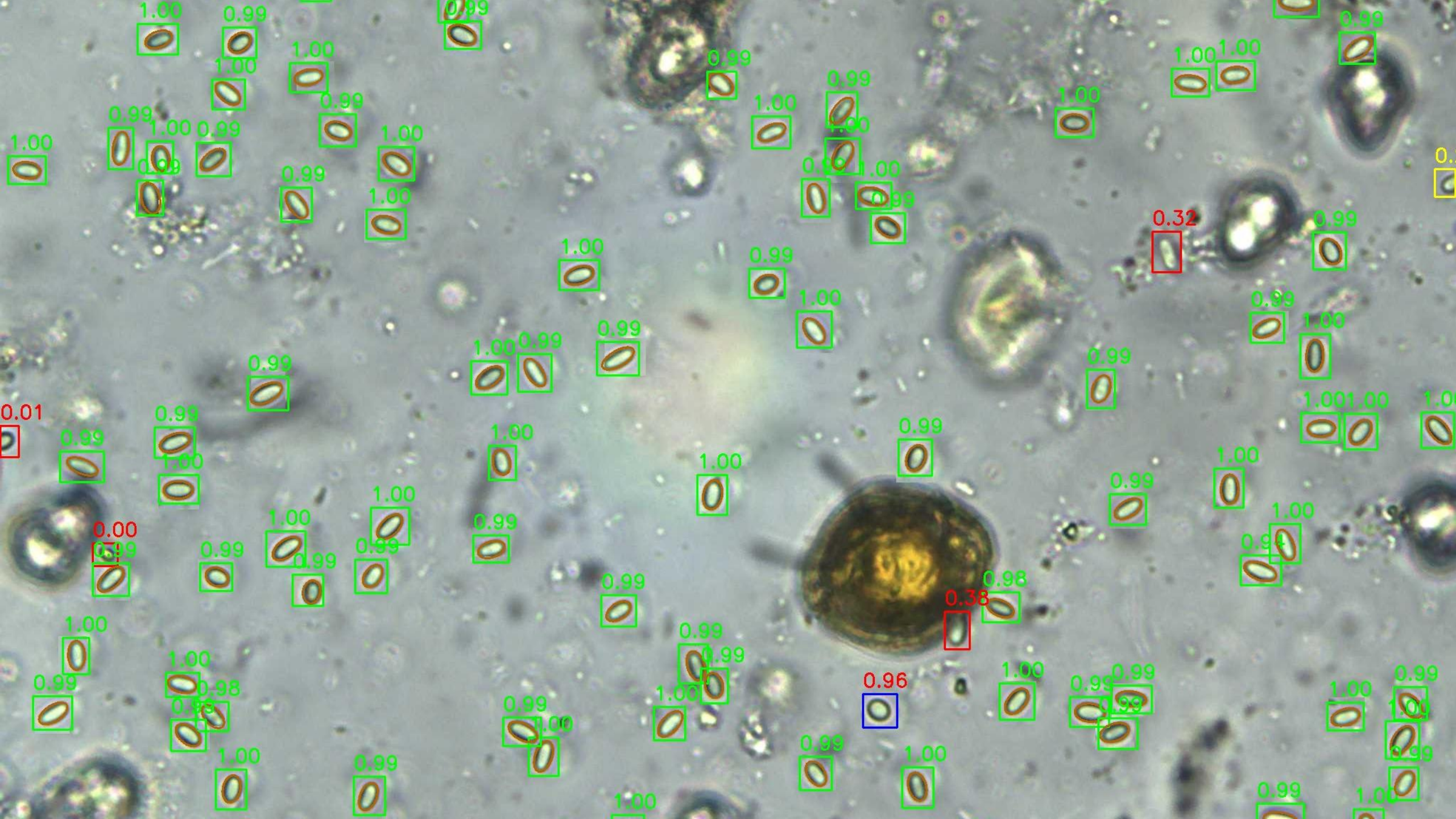
Sample preparation
(>30 adult bees)

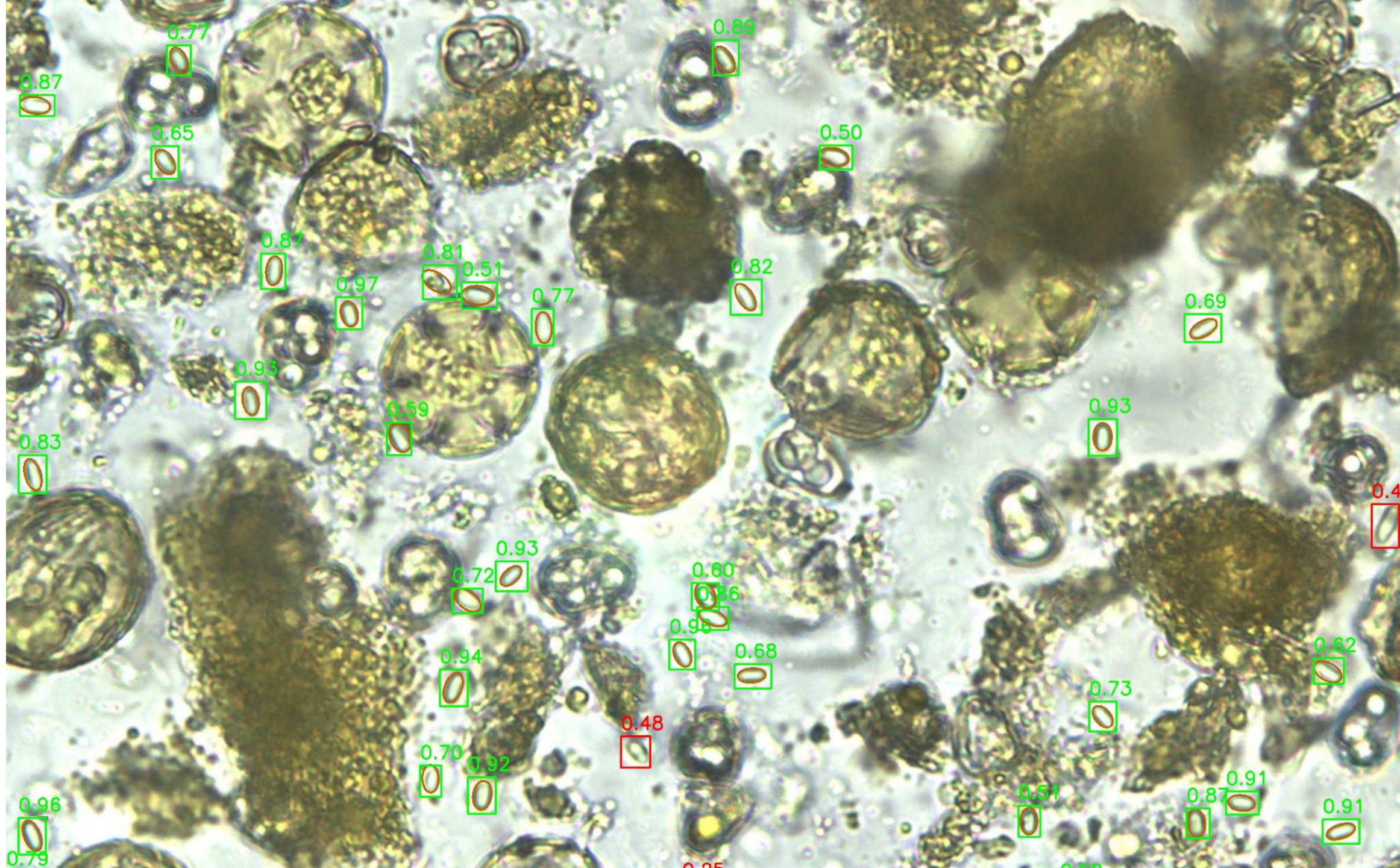
Sample placement and
launch of test

Sample scanning and
cloud upload
and process

PDF
Generation for
results









NosemAI. 1st Accuracy validation

- 50 samples analysed with:
 - NosemAI
 - q-PCR Dúplex of *N. Ceranae* and *N. Apis*
 - *Traditional counting microscope*



CONCLUSIONS:

- **0.95 correlation** between manual and AI-based counting
- **PCR showed lower reliability in terms of quantitative technique**
- **For APIS/CERANAE differentiation, needed better confirmation with Apis Samples.**
- **More sensitive technique for low loads below $1 \cdot 10^4$**



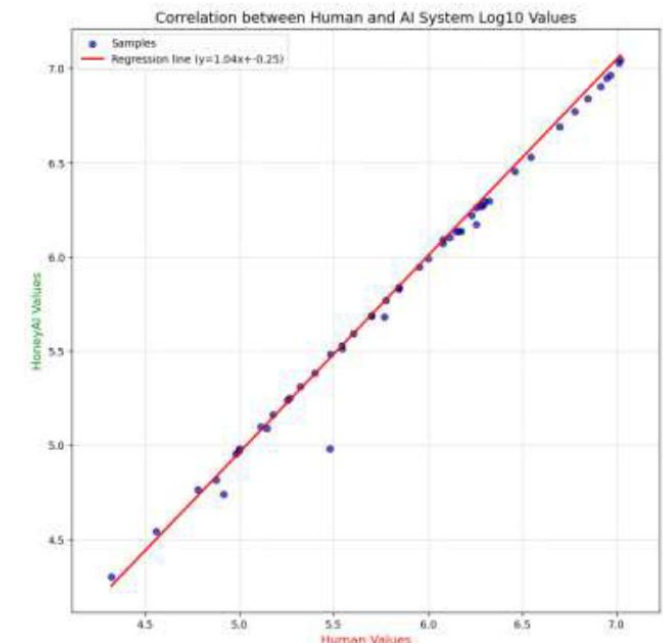
NosemAI. 2nd Accuracy validation

- 53 real samples analysed with:
 - NosemAI – duplicate analysis
 - *Traditional counting microscope (20 squares Neubauer chamber)*



CONCLUSIONS:

- **Pearson Correlation coefficient (r): 0.9942**
- **Coefficient of determination (R^2): 0.9884**
- **For APIS/CERANAE differentiation, still it is needed better confirmation with Apis Samples.**
- **For samples with weaker infestations, the HoneyAI application demonstrated better accuracy and effectiveness compared to conventional haemocytometer counting methods**



Datasets creation

Malpighamoeba cysts

Malpighamoeba mellificae is a single-celled protozoan parasite that infects the Malpighian tubules of adult honeybees (*Apis mellifera*), causing the contagious disease **amoebiasis**.

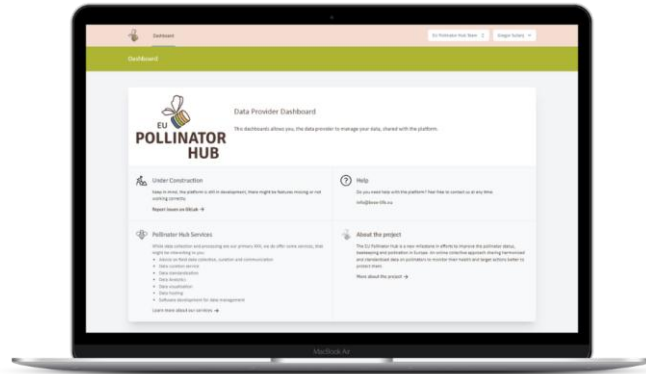
Usually, nosemosis and amoebiasis infestation co-exists.

The organism measures 3-15 μm in size, with cysts typically appearing as spherical, colorless, translucent structures of 2,5 - 8 μm diameter.





NosemAI. Benefits for Apiculture



<https://pollinatorhub.eu/>



100%

Automated sample scanning
and detection



10 min

Results ready in less than 5
minutes



Useful

Very useful to increase bee
health controls'



Quantitative

Technique



< € 10 per test

Very affordable



Development Project – Thanks to:



ACCIÓ  Generalitat de Catalunya

 **Funded by
the European Union**
NextGenerationEU



Grant agreement
No. FENG.02.23-IP.01-0001/23-00
The project is co-financed by the
European Union's Digital Europe
Framework Programme
contract no. 101100622.



Validation Project – Thanks to:



THANK YOU!



www.honey-ai.com



Iratxe.perales@microfy.ai



LinkedIn: Honey.AI