

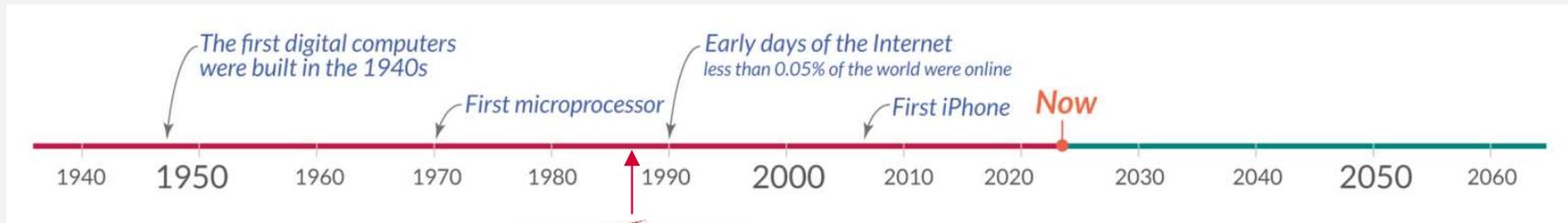
AI ASSISTED DIGITAL MICROSCOPY IN MICROBIOLOGY

Mert Corbaci, PhD
DNN Applications Engineer
MetaSystems Group, Inc.

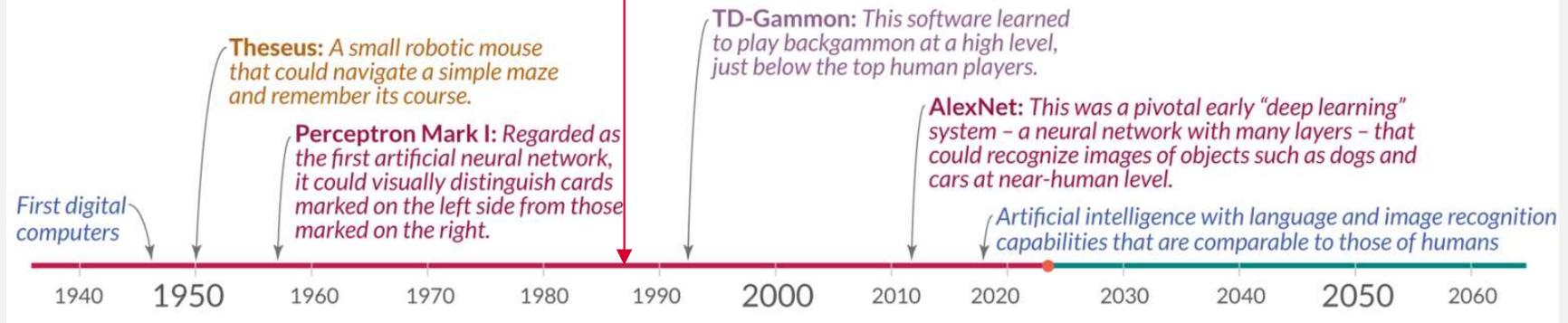
AI ASSISTED DIGITAL MICROSCOPY IN MICROBIOLOGY

- MetaSystems
- Automation in Microbiology and Microscopy
- Challenges & Limitations
- AI Assisted Microscopy
- Benefits, Challenges, Limitations

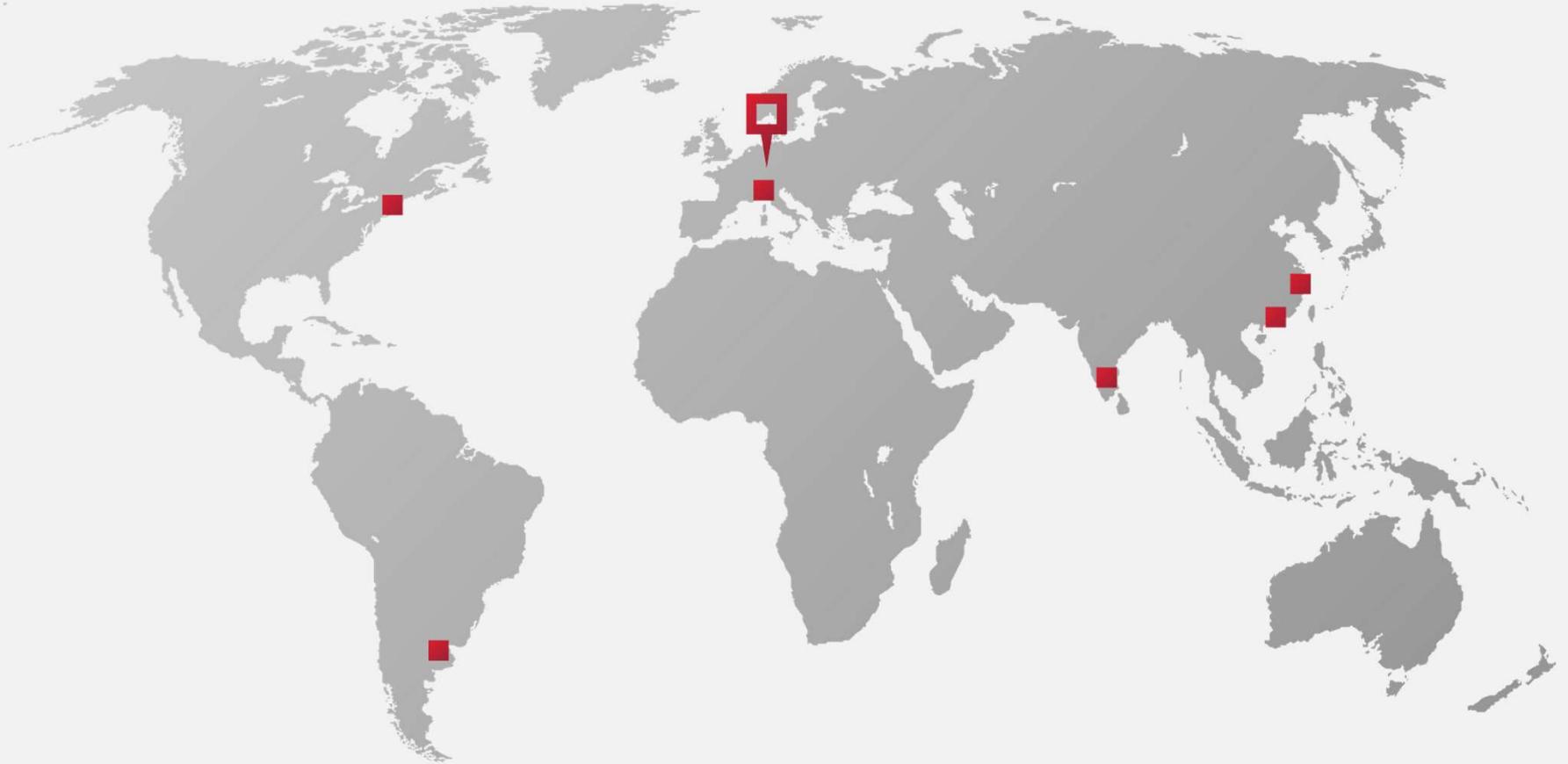
COMPUTERS & ARTIFICIAL INTELLIGENCE



A timeline of notable artificial intelligence systems



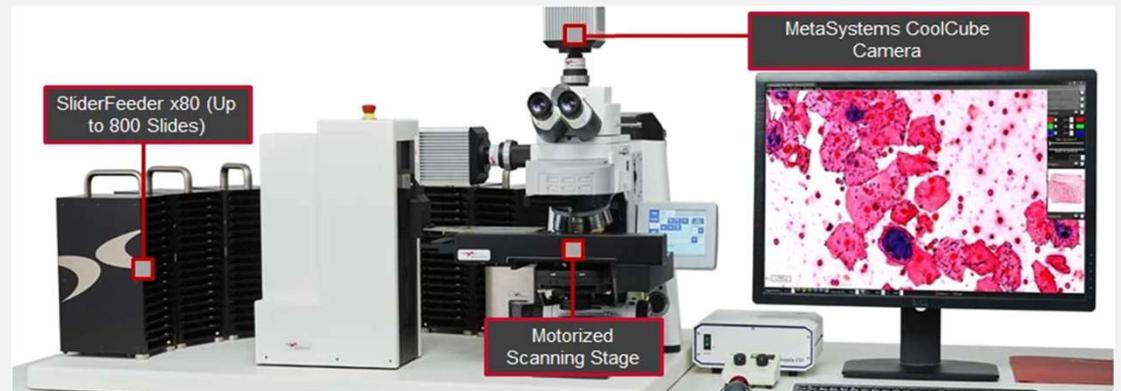
METASYSTEMS



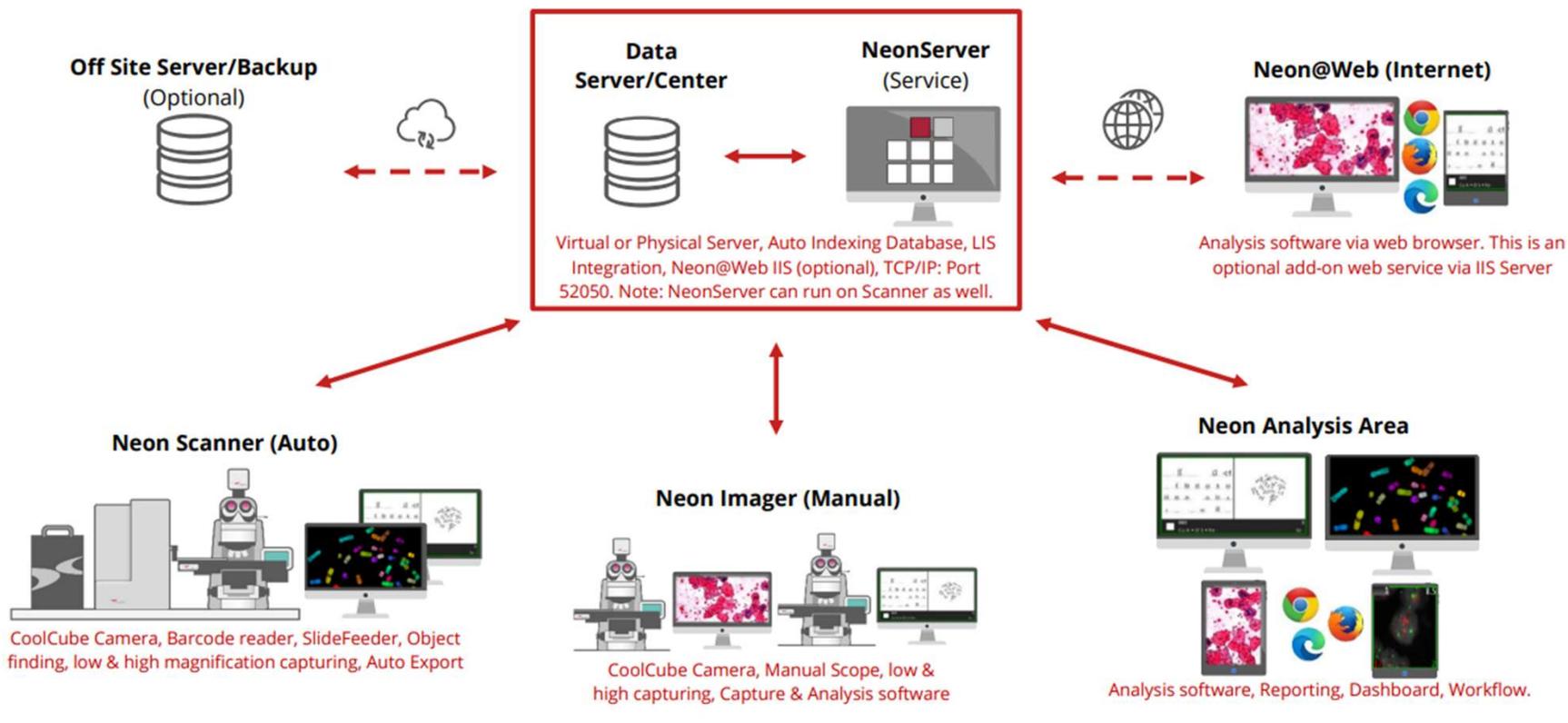
A BRIEF HISTORY

- **1983 – How to Find Metaphases**
- **1985 – Metafer**
- **1986 – MetaSystems**
- **1991 – Ikaros (Karyotyping software)**
- **1995 – MetaSystems Boston Office**
- **2002 – High throughput**
- **2010 – Whole Slide Stitching**
- **2013 – Collaboration with Copan, first microbiology application**
- **2016 – Neon Database Management Software**
- **2021 – Patented AI Assisted Karyotyping**

METASYSTEMS SCANNER



High Level Architecture



DIGITAL MICROBIOLOGY

Aspects of digital microbiology in the diagnostic process

| Process | Aspect | Example |
|----------------|--------------------------|--|
| Pre-analytics | Quality control | <p>What is the sample quality?</p> <ul style="list-style-type: none"> – Automated measurement and feedback regarding the correct filling of blood culture bottles. – Automated assessment of sample contamination including species and clinical score |
| | Diagnostic stewardship | <p>Which additional diagnostic test should be ordered?</p> <ul style="list-style-type: none"> – Suggestion based on a digital twin, smartphone app, or chatbot |
| Analytics | Quality control | <p>How reliable is the analytical performance of a test?</p> <ul style="list-style-type: none"> – Surveillance of reagent lots performance with internal and external controls and automated reporting in connection to specific used lots of time |
| | Imaging | <p>Are there bacteria on the microscope slide?</p> <ul style="list-style-type: none"> – Automated image acquisition with a microscope and scan for pathogen-like structures and category |
| | Plate reading | <p>Is there bacterial growth on the plate?</p> <ul style="list-style-type: none"> – Automated image acquisition and scan for colonies and subsequent identification (telebacteriology) |
| | Expert system | <p>Does the detected resistance profile make sense?</p> <ul style="list-style-type: none"> – Medical validation of antibiotic resistance profiles with expert database |
| | Public Health | <p>Is there a potential outbreak?</p> <ul style="list-style-type: none"> – Automated screening for pathogen similarities, e.g., resistance profile or automated bioinformatics |
| Post-analytics | Highlight important data | <p>Is there a potential bacterial phenotype?</p> <ul style="list-style-type: none"> – Detection of resistance by analysing MALDI-TOF spectra |
| | Sepsis treatment | <p>What is the best treatment for the patient?</p> <ul style="list-style-type: none"> – Prediction of sepsis, and best treatment, e.g., volume and antibiotics for the patient |

Source: Egli, A., Schrenzel, J., & Greub, G. (2020). Digital Microbiology. *Clinical Microbiology and Infection*, 26(10), 1324–1331. <https://doi.org/10.1016/j.cmi.2020.06.023>

CHALLENGES OF AUTOMATION

HISTORICAL IMPEDIMENTS TO AUTOMATION IN MICROBIOLOGY

- Microbiology is too complex to automate.
 - Specimen types: blood, sterile body fluids, tissues, urine, catheter tips, other prosthetic devices, respiratory tract specimens
 - Transportation, variety of vessels
 - Processing: concentrated, macerated, digested, decontaminated, sonicated prior to being plated, or plated directly;
 - plating can be quantitative, semiquantitative, or nonquantitative
- No machine can replace a human in the microbiology laboratory.
 - Machines cannot operate fast enough
 - Decision-making, critical-thinking
- Cost of automation can be too high.
- Microbiology laboratories are too small for automation.

Bourbeau, P. P., & Ledebor, N. A. (2013a). Automation in clinical microbiology. *Journal of Clinical Microbiology*, 51(6), 1658–1665.
<https://doi.org/10.1128/jcm.00301-13>

CHALLENGES OF AUTOMATION

HISTORICAL IMPEDIMENTS TO AUTOMATION IN MICROBIOLOGY

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- Cost of automation.
- Microbiology laboratories are too small for automation.

Driving forces for automation

- Increasing test volumes
- 24/7 labs
- Staff shortages
- Advancement of technology

Bourbeau, P. P., & Ledebor, N. A. (2013a). Automation in clinical microbiology. *Journal of Clinical Microbiology*, 51(6), 1658–1665.
<https://doi.org/10.1128/jcm.00301-13>

WHOLE > Σ (*PARTS*)

- Sample collection
- Accessioning
- Sample preparation
- Processing
- Data acquisition
- Analysis/identification
- Resulting/reporting

WHOLE > Σ (PARTS)

- Sample collection
- Accessioning
- Sample preparation
- Processing
- Data acquisition
- Analysis/identification
- Resulting/reporting



Interoperability
&
Standardization

WHOLE > Σ (PARTS)

- Sample collection
- Accessioning
- Sample preparation
- Processing
- Data acquisition
- Analysis/identification
- Resulting/reporting



Interoperability
&
Standardization



Quality
Accuracy
Speed



Better work environment
Better healthcare
Lower cost

DIGITAL MICROSCOPY

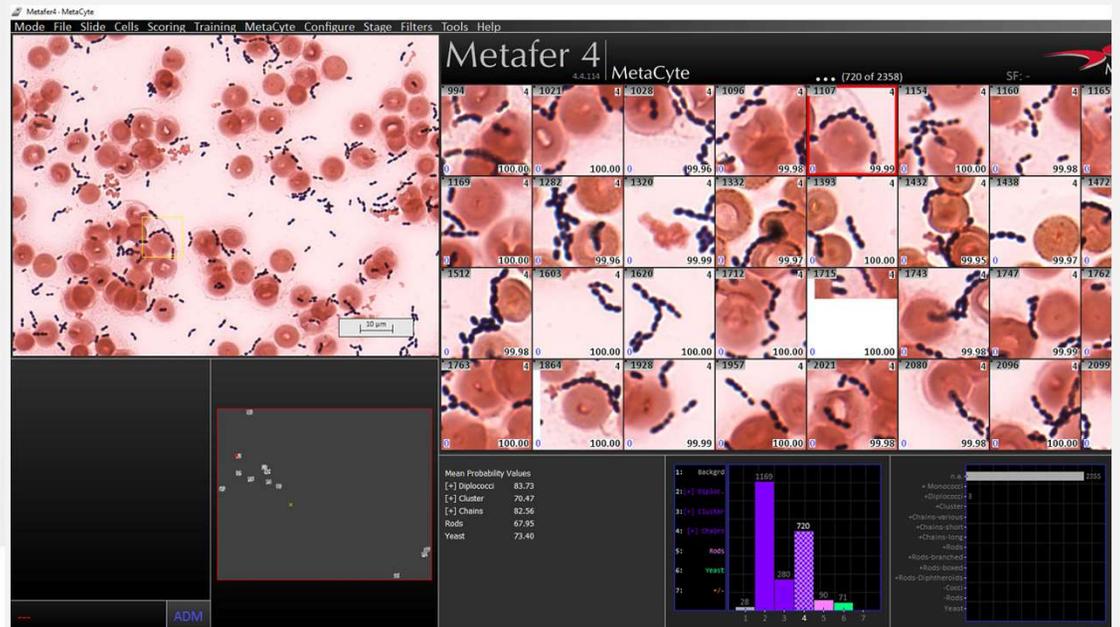
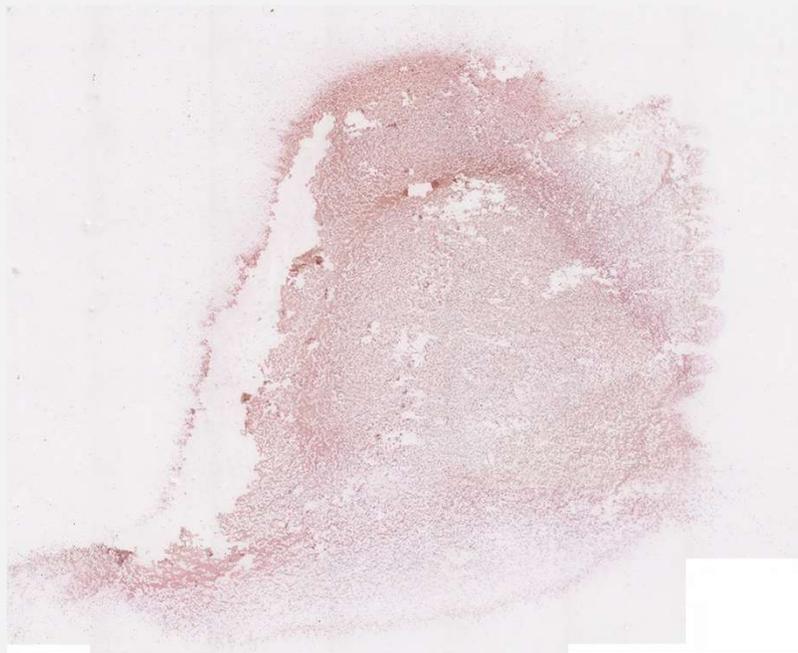
- Data acquisition
- Storage
- Quality control
- Analysis
- Security and protection
- Interoperability/interconnection
- Reporting and visualization

- Hardware
- Software
- Infrastructure
- Cybersecurity

- 12 MP images
- Multiple focus planes (z-stack)
- Imaging 3 mins average

- ~ 500 MB – 1 GB raw data per slide
- Containing patient demographics

DIGITAL MICROSCOPY



DIGITAL MICROSCOPY

2023-09-24 21:40

Ikaros Neon 6.3.3 Report More ADM Microbiology MetaSystems

Dashboard

Cases

Search ...

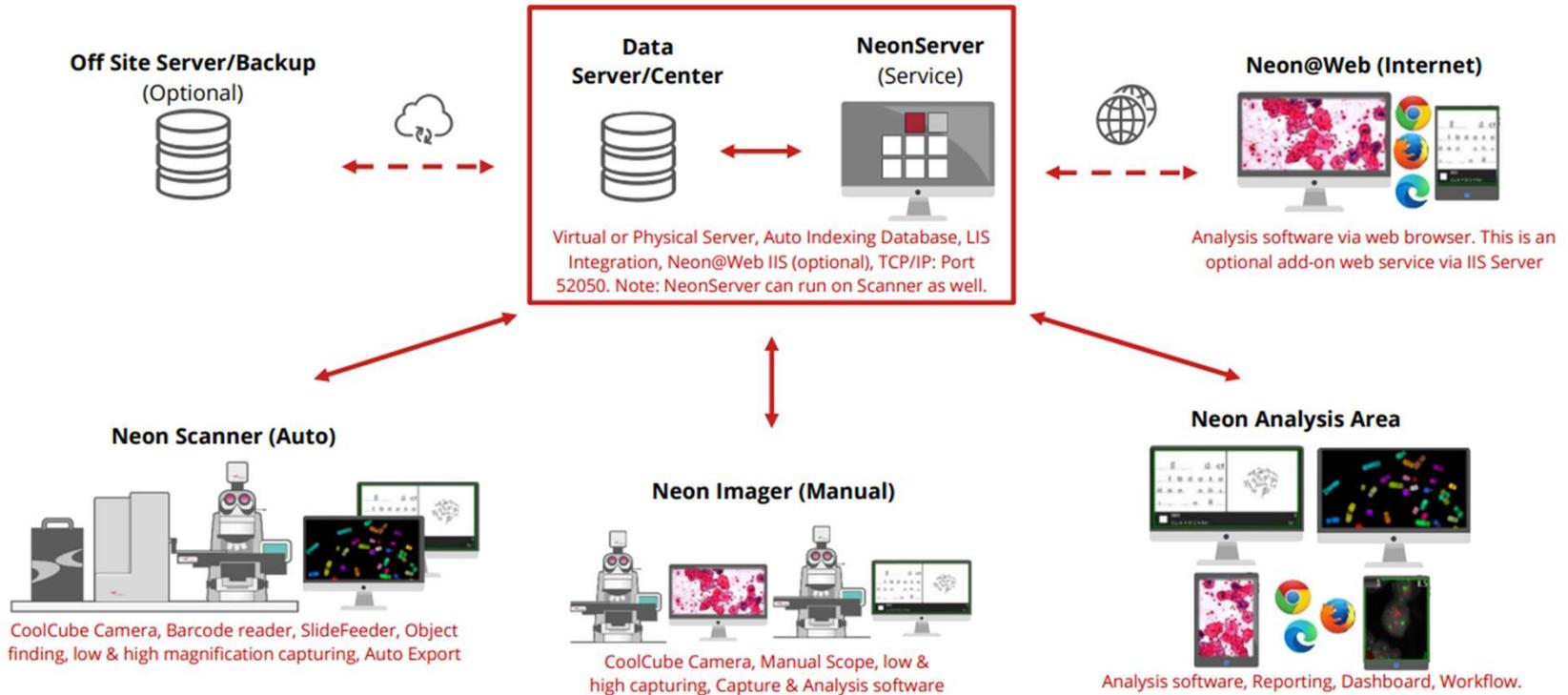
State Department Specimen Virtual Slides

| Case | State | Department | Specimen | Created | Scans | Result | Comment | Patient No | Birthdate | Checked |
|---------------|-----------|--------------|----------|---------------------|-------|--------------|--------------------------------|------------|------------|---------|
| OPLH2018 | Created | | | 10/20/2020 13:43:01 | 0 | | | 483968 | 10/23/1980 | |
| OPLH2012 | Captured | Parasitology | | 10/20/2020 13:42:04 | 1 | | | 475839 | 07/13/1978 | |
| OPLH2020 | Reviewed | | | 10/20/2020 08:17:38 | 0 | | | 5463786 | 08/20/1977 | |
| OPLH2019 | Captured | Parasitology | Stool | 10/20/2020 08:16:00 | 1 | | | 567497 | 06/14/1966 | |
| OPLH2007 | In Review | Parasitology | Stool | 10/20/2020 08:13:32 | 1 | O&P Positive | | 476893 | 10/17/1979 | |
| GR-MGI-060801 | Captured | Bacteriology | | 06/08/2020 12:40:54 | 2 | | | | | |
| OP204564 | Captured | Parasitology | Stool | 05/26/2020 14:52:53 | 1 | | | 563258 | 05/26/1976 | |
| OP204585 | In Review | Parasitology | Stool | 05/26/2020 14:52:53 | 1 | OP Positive | Giardia | 563258 | 05/26/1976 | |
| OP204569 | Captured | Parasitology | Stool | 05/26/2020 12:51:55 | 1 | | D. Frag. | 466415 | 03/10/1963 | |
| OP204583 | Captured | Parasitology | Stool | 05/26/2020 12:51:55 | 1 | | | 466415 | 03/10/1963 | |
| GR204065 | Captured | Bacteriology | Wound | 05/26/2020 10:35:19 | 2 | | G+ Cocci, G- rods, VSlide | 828961 | 04/01/1989 | |
| AR204863 | Captured | Mycobacteria | | 05/22/2020 17:21:21 | 1 | | AuramineO - S | 394603 | 05/26/1985 | |
| GRD20492 | In Review | Bacteriology | Sputum | 05/22/2020 16:49:18 | 1 | | DNN G+ Diplococci, G+ Clusters | 484207 | 04/04/1964 | |
| ZN204869 | In Review | | | 05/22/2020 11:11:03 | 1 | | Ziehl-Neelsen | 526348 | 05/26/1970 | |
| AR204869 | In Review | Mycobacteria | | 05/22/2020 11:07:11 | 1 | | AuramineO - S | 457658 | 02/01/1984 | |
| OP209467 | Checked | Parasitology | Stool | 05/22/2020 10:32:10 | 1 | O&P Positive | Blasto, E. Nana | 865395 | 12/05/1979 | ADM |
| OP209494 | Checked | Parasitology | Stool | 05/22/2020 10:32:10 | 1 | | | 865395 | 12/05/1979 | ADM |
| AR208586 | Captured | Mycobacteria | | 05/22/2020 10:25:30 | 1 | | AuramineO - W | 480716 | 12/05/1960 | |
| GR204593 | Checked | Bacteriology | Blood | 05/22/2020 09:56:12 | 2 | | G+ Cocci Chains | 157691 | 05/22/1981 | ADM |
| 185018625012 | In Review | | Blood | 05/22/2020 09:54:05 | 2 | | G+ cocci short chains | 361063 | 07/17/1968 | |
| GR200395 | In Review | Bacteriology | Blood | 05/22/2020 09:47:31 | 2 | | G+ Cocci Chains | 798523 | 06/13/1979 | |
| OP205772 | Checked | Parasitology | Stool | 05/21/2020 13:23:08 | 1 | O&P Positive | D. Frag. | 751682 | 07/05/1965 | ADM |
| HS20003363 | In Review | Cyto | | 03/02/2020 14:57:56 | 4 | | | | 06/08/1947 | |

500 / 500 14 148 197 137 4

Open Case Ikaros / Isis Delete Rename Copy Other

High Level Architecture



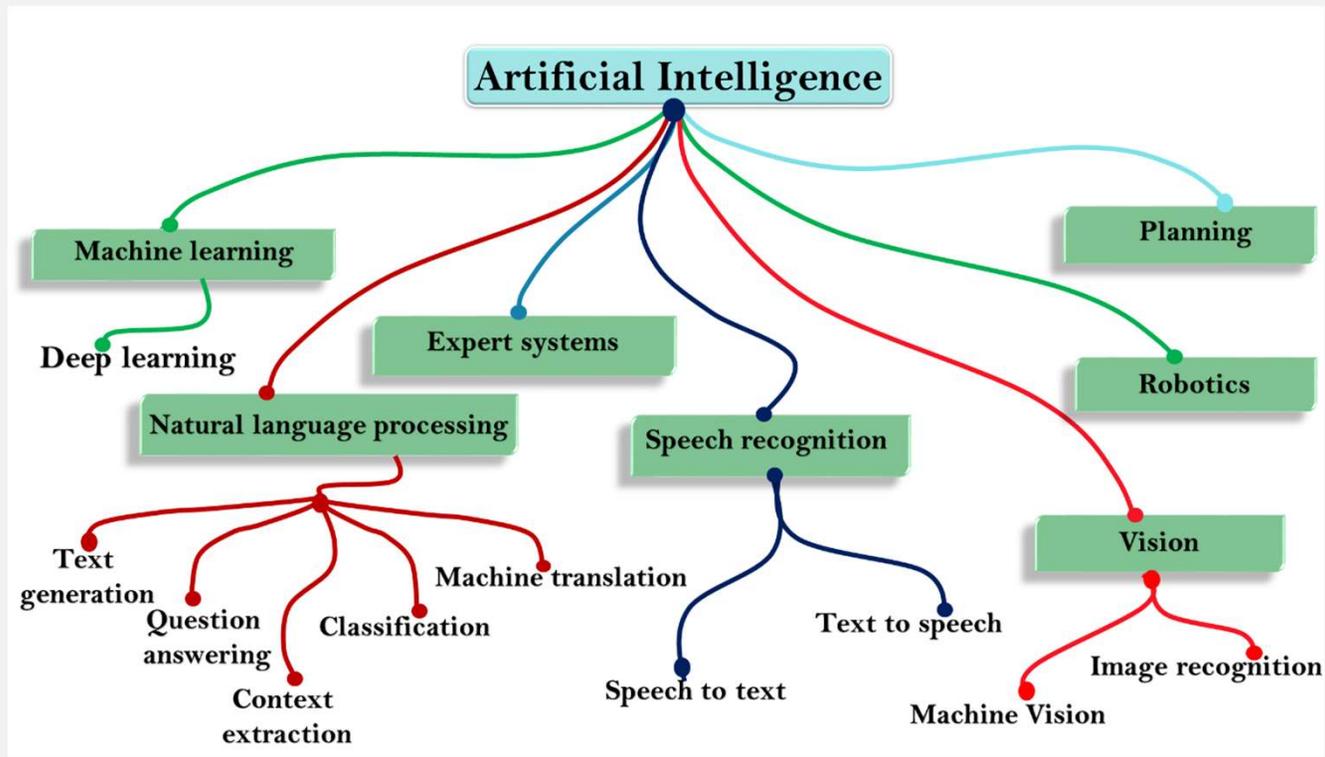
ARTIFICIAL INTELLIGENCE

ARTIFICIAL INTELLIGENCE (AI)

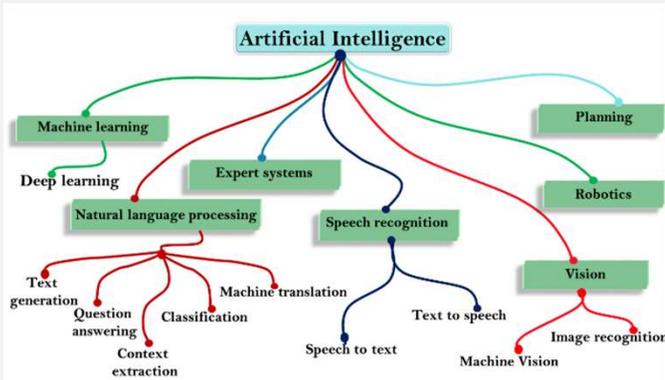
- ❑ Computer systems able to perform tasks that normally require human intelligence (visual perception, speech recognition, translation between languages, decision-making etc.)
- ❑ Theory and development of computers systems able to perform tasks that normally require human intelligence
- ❑ Computer Science, Statistics, Neuroscience, Psychology



ARTIFICIAL INTELLIGENCE (AI)



ARTIFICIAL INTELLIGENCE (AI)

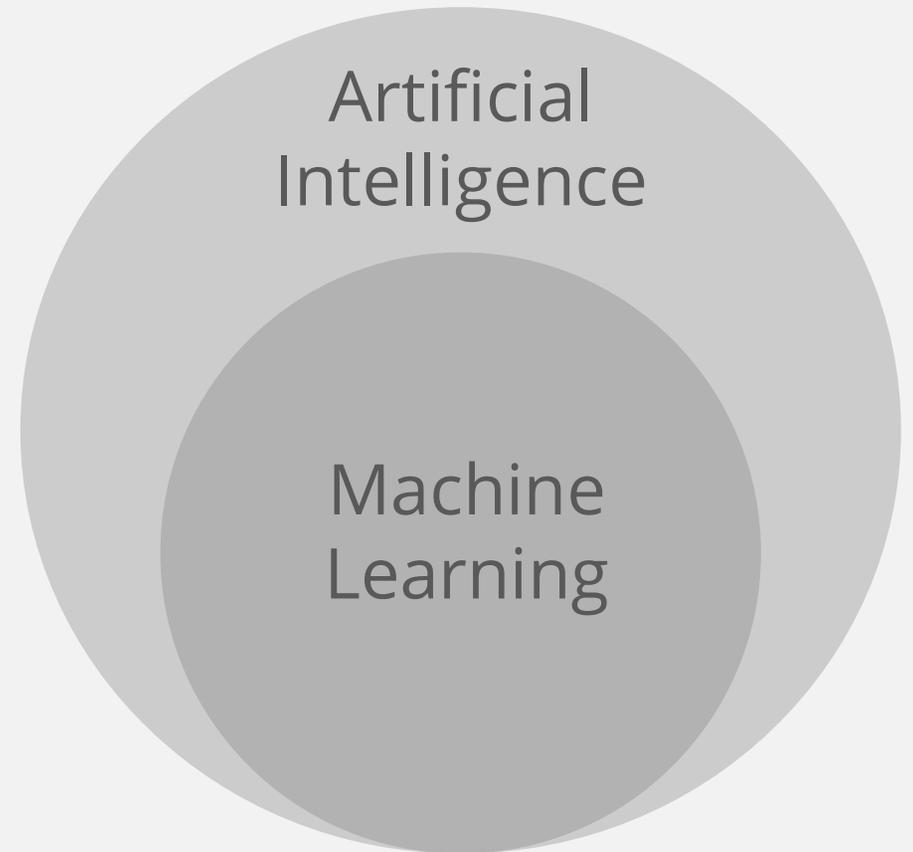


Aspects of digital microbiology in the diagnostic process

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MACHINE LEARNING (ML)

- ❑ Computation learning using algorithms to learn from and make predictions on data
- ❑ Gives computers the ability to learn without bring explicitly programmed
- ❑ the use and development of computer systems that are able to learn and adapt without following explicit instructions, by using algorithms and statistical models to analyze and draw inferences from patterns in data.
- ❑ **In classical machine learning, an expert manually designs features that can be used to distinguish objects-of-interest by different parameters, e.g., shape, color, or texture. In this process called feature engineering, the expert transfers knowledge to the algorithm.**



DEEP LEARNING

Artificial Intelligence

Enables a machine to mimic human behavior

Machine Learning

Distinguishing features engineered by human developer

Deep Learning

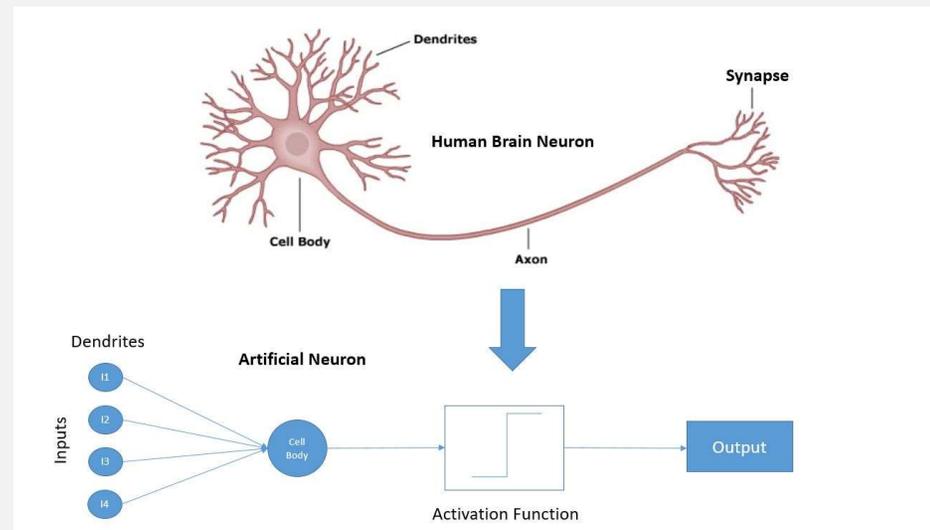
Distinguishing features acquired by Deep Neural Network (DNN)



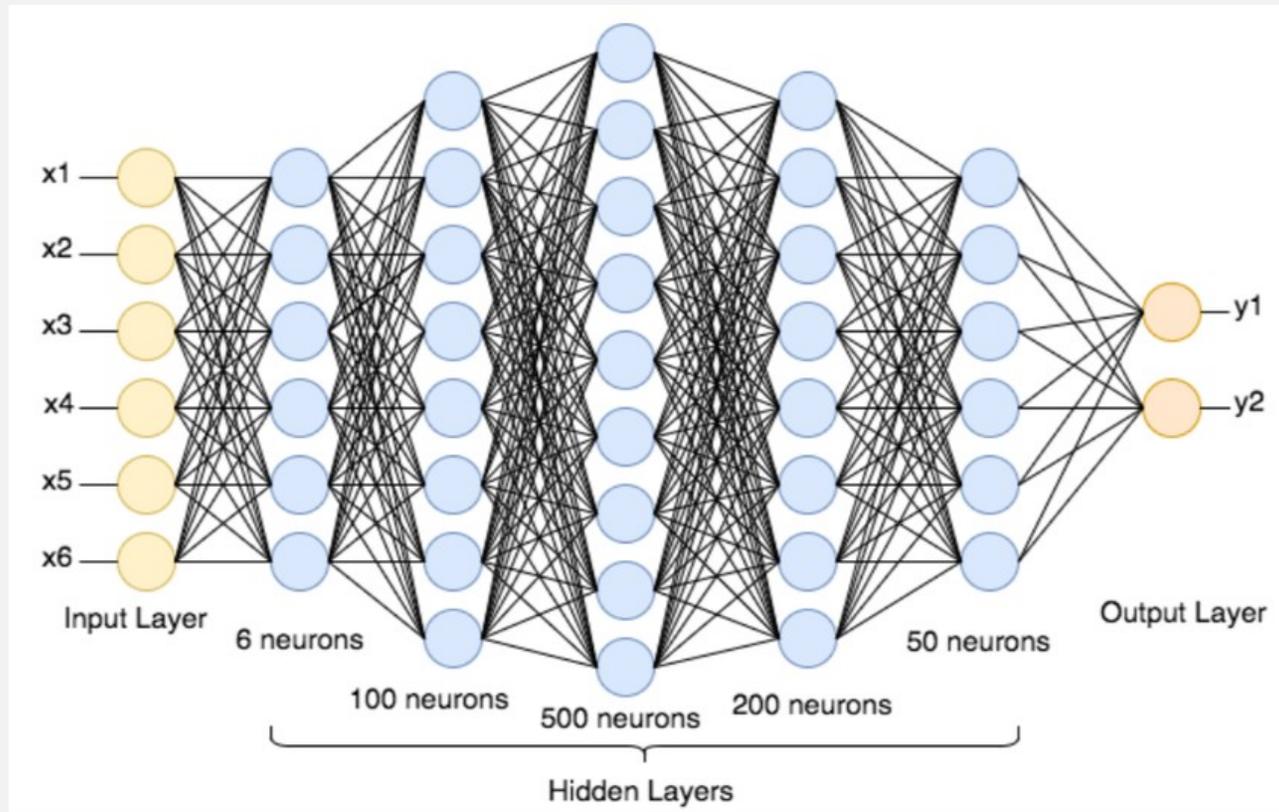
Machine learning algorithms with brain-like logical structure of algorithms called artificial neural networks



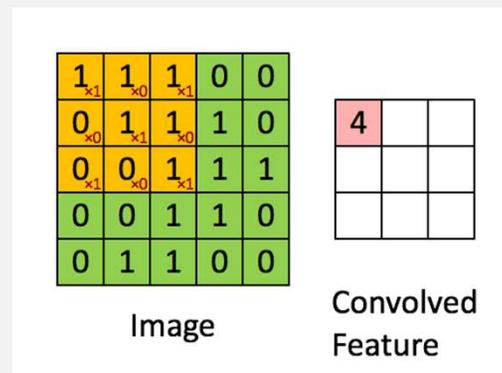
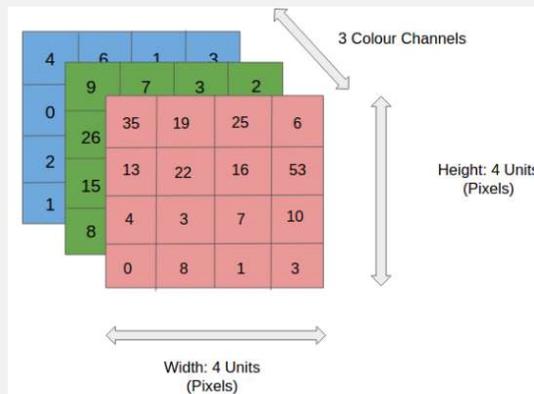
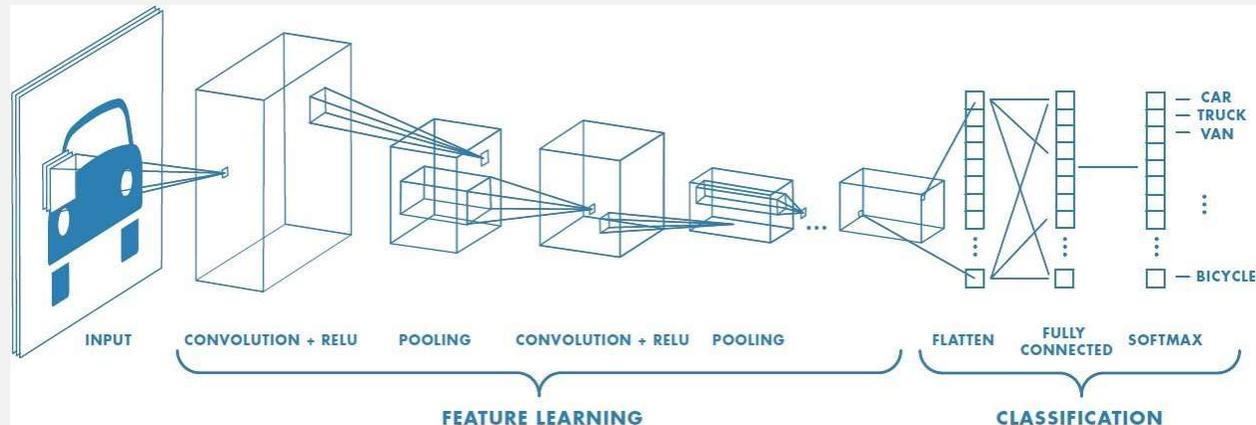
Biomimetics



DEEP LEARNING



DEEP LEARNING



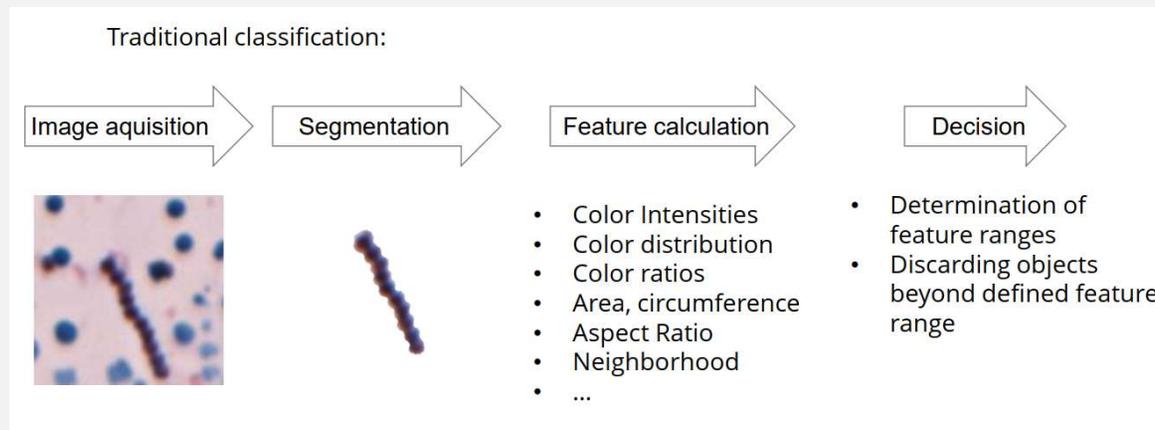
MACHINE LEARNING

Traditional classification:

Feature engineering

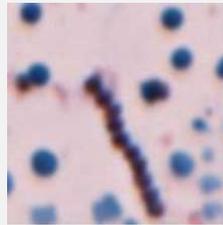
- Dark
- Elongated
- Made of small round objects
- Certain size
- ...

The programmer “describes” the object, transfers knowledge to the algorithm.



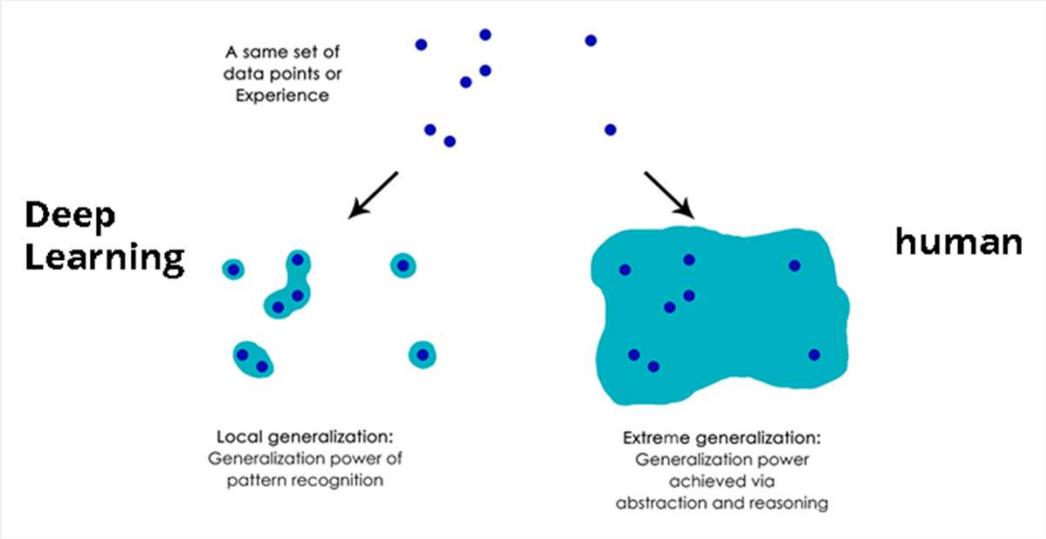
DEEP LEARNING

Deep Learning:
“Neuronal net” just learns that THIS image HAS TO BE “Gram-positive Chain” = Learning by examples.



Problem: It does not know why. Is it the dark Rod? The blue clouds? The pink background? The noise?

AI-ASSISTED DIGITAL MICROSCOPY



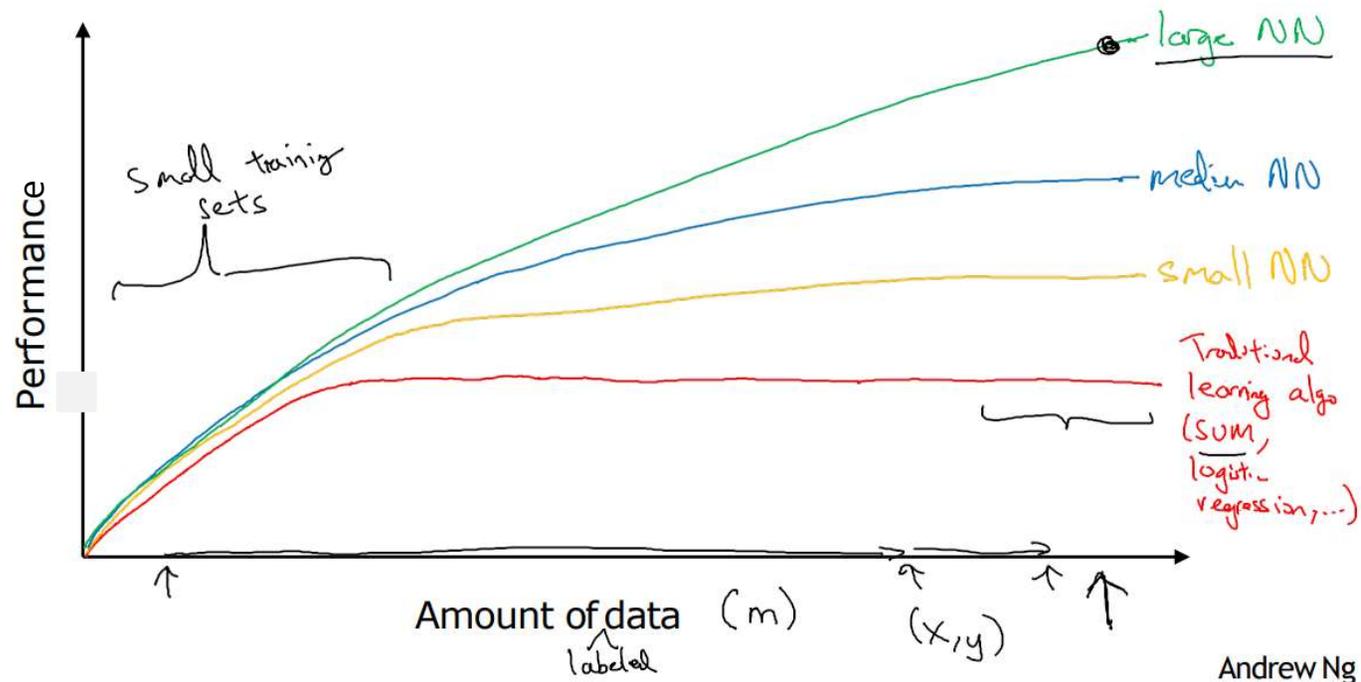
AI-ASSISTED DIGITAL MICROSCOPY

- **Solution:** Show a huge amount of example images of the object in all variations. This is the main reason why deep learning needs **big data!**
- The “intelligence” **comes from the people** who initially labeled all the images
- This has to be done for every class!



WHY NOW?

Scale drives deep learning progress



Source: deeplearning.ai

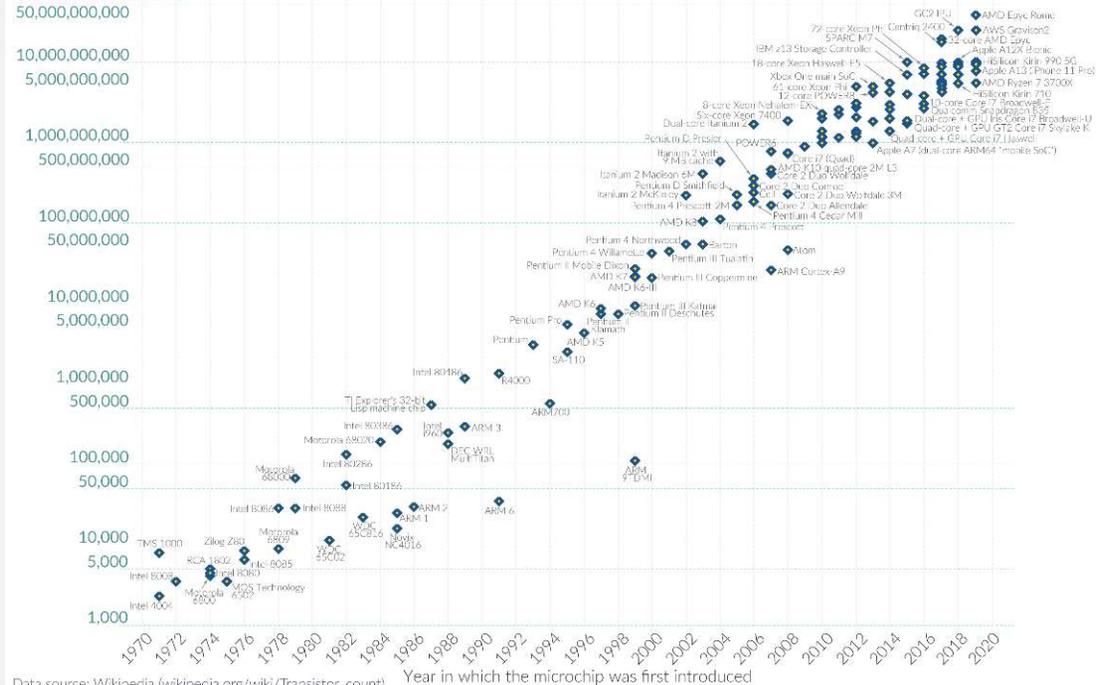
WHY NOW?

Moore's Law: The number of transistors on microchips doubles every two years

Our World
in Data

Moore's law describes the empirical regularity that the number of transistors on integrated circuits doubles approximately every two years. This advancement is important for other aspects of technological progress in computing – such as processing speed or the price of computers.

Transistor count



Data source: Wikipedia (wikipedia.org/wiki/Transistor_count)
 OurWorldInData.org – Research and data to make progress against the world's largest problems. Licensed under CC-BY by the authors Hannah Ritchie and Max Roser.

HARDWARE

- CPU: Central Processing Unit
 - Basic arithmetic, logic, input/output processes specified by the instructions in a program
- Processor Core: an individual processor within a CPU



HARDWARE

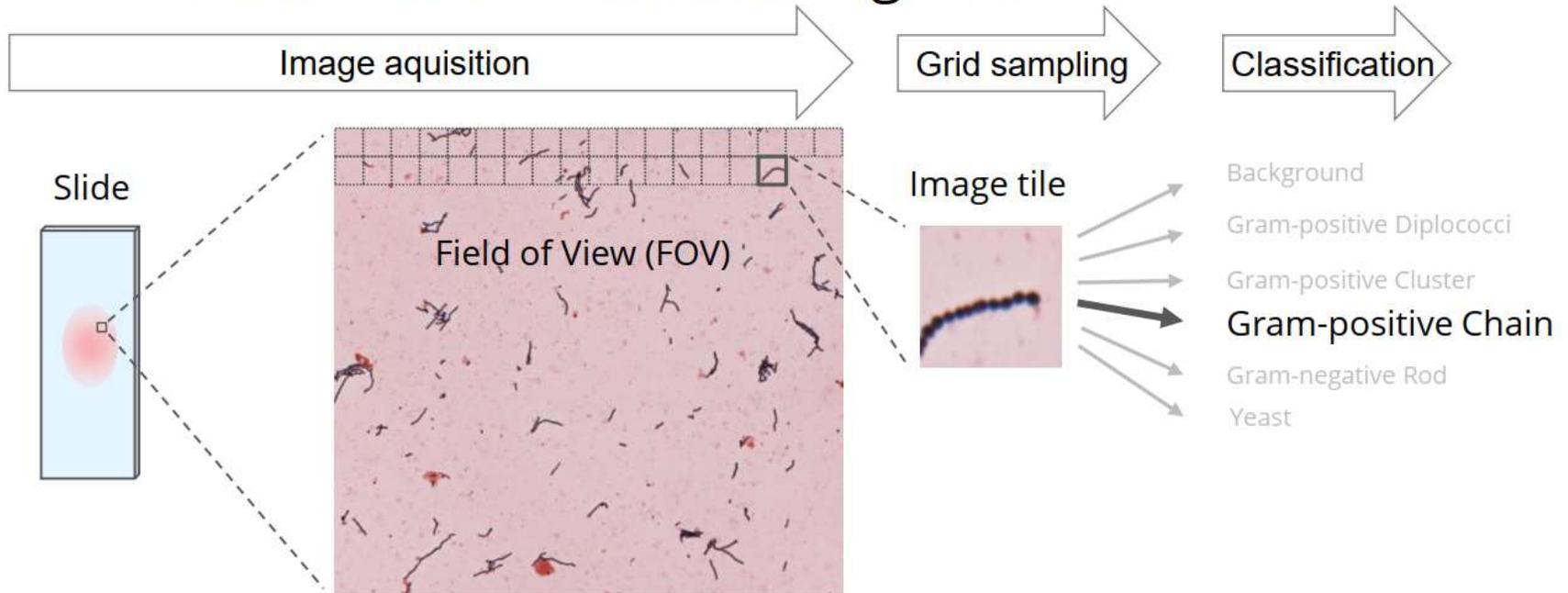
- GPU: Graphics Processing Unit
- CUDA: Compute Unified Device Architecture (NVIDIA)
(AMD's "Stream Processors")
 - is a specialized programming language that can leverage the GPU in specific ways to perform tasks with greater performance

| | <u>RTX 2080</u> | <u>GTX 1650</u> |
|------------------------|-----------------|-----------------|
| Pipelines / CUDA cores | 2944 | 896 |
| Number of transistors | 13600 million | 4700 million |



AI-ASSISTED DIGITAL MICROSCOPY

General DNN workflow without segmentation:
Field of View is cut in image tiles



AI-ASSISTED DIGITAL MICROSCOPY

Why automation?

- ▣ Less hands-on microscope time
- ▣ Higher sensitivity
- ▣ Faster results

Challenges for imaging & analysis

- ▣ Focusing
 - ▣ Negative slides
 - ▣ Thick/dense samples
- ▣ Stain/slide prep consistency

AFB (AURAMINE-O STAIN)

Metafer4 - MetaCyte

Mode File Slide Cells Scoring Training MetaCyte Configure Stage Filters Tools Help

Metafer 4
4.3.127
MetaCyte

MetaSystems

Results
Results only valid after expert review!
 (Automatic rejection at probability of 50 %)
 positive Field of View
 Tiles 20x Camera 40x Ocular
 795 210 293
 795 70 98
 339 7 10
 68 1 1.4
 Grading: total Number of scanned FOV:
 ++++ 136 190

Setup
 Search
 Gallery
 Field Review 2
 Ikaros
 Exit

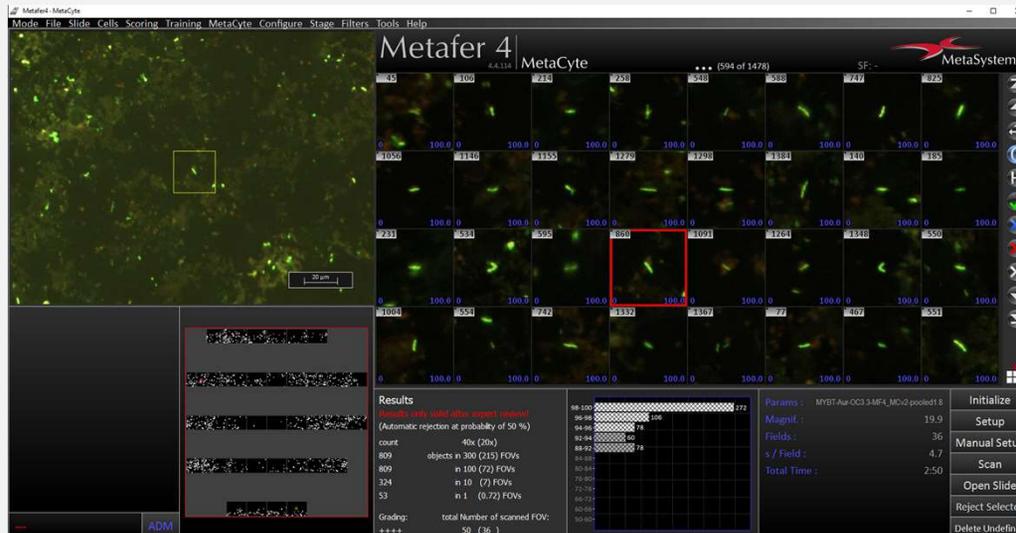
AR204863 4696 795 0 146 3755 ???
 GR208694~B
 AR204863 4696
 OP205486~B 4760

AFB (AURAMINE-O STAIN)

The screenshot displays the Metafer 4 software interface. The main window shows a grid of 10x10 fluorescence microscopy images of AFB (Auramine-O stain). The top-left image is a larger view with a yellow box highlighting a region. The bottom-left image shows a zoomed-in view of the highlighted region with a red box. The interface includes a menu bar (Mode, File, Slide, Cells, Scoring, Training, MetaCyte, Configure, Stage, Filters, Tools, Help), a title bar (Metafer 4 - MetaCyte), and a status bar (MetaSystems). The main window displays the following information:

- Metafer 4 | MetaCyte** (4.4.114)
- MetaSystems** logo
- Grid of Images:** A 10x10 grid of images. The top row contains image IDs: 45, 106, 214, 258, 548, 588, 747, 825. The bottom row contains image IDs: 1004, 554, 742, 1332, 1367, 77, 467, 551.
- Results:**
 - Results only valid after expert review!** (Automatic rejection at probability of 50 %)
 - count: 40x (20x)
 - 809 objects in 300 (215) FOVs
 - 809 in 100 (72) FOVs
 - 324 in 10 (7) FOVs
 - 53 in 1 (0.72) FOVs
 - Grading: total Number of scanned FOV: 50 (36)
- Params:** MYBT-Aur-OC3.3-MF4_MCV2-pooled1.8
- Magnif.:** 19.9
- Fields:** 36
- s / Field:** 4.7
- Total Time:** 2:50
- Control Panel:** Initialize, Setup, Manual Setup, Scan, Open Slide, Reject Select, Delete Undefined

AFB (AURAMINE-O STAIN)



Requirements for automation:

- Focusing on negative slides
 - Use of counter-stain
 - Slides with printed/etched patterns
- Consistent staining
- Consistent slide prep

OVA & PARASITE

Metafer 4 - MetaCyte

Mode File Slide Cells Scoring Training MetaCyte Configure Stage Filters Tools Help

Metafer 4
4.3.127
MetaCyte
... (137 of 4760)

MetaSystems

O&P
Trichrome
DNN Analysis

ADM

| Class | Count |
|-------------------|-------|
| Background | 0 |
| Yeast | 0 |
| Parasit | 0 |
| Chlamy Lymph Cell | 0 |
| White Blood Cell | 0 |
| Red Blood Cell | 0 |
| Droplet | 0 |
| Fungal Candida | 0 |
| Bleed | 0 |
| O. Fragilis | 0 |
| E. Nuclei | 0 |
| E. Fragment | 0 |
| E. Trophozoite | 0 |
| E. Cell | 0 |
| Oocyst | 0 |
| Cybe | 0 |
| Isosporidia | 0 |
| Cell Group JB | 0 |

Artifacts 2748
Other Negatives 150
Blasto 1623
Positive 137 8665

Setup
Search
Gallery
Field Review 2
Ikaros
Exit

OVA & PARASITE

Metafer 4 - MetaCyte

Mode File Slide Cells Scoring Training MetaCyte Configure Stage Filters Tools Help

Metafer 4
4.3.127
MetaCyte
... (137 of 4760)

MetaSystems

The interface displays a grid of 80 microscopic images (8x10) with a larger image on the left. A yellow box highlights a specific cell in the large image. The bottom right corner features a classification summary table:

| Class | Count |
|-------------------|-------|
| Background | 0 |
| Yeast | 0 |
| Parasit | 0 |
| Chlamy Lymph Cell | 0 |
| White Blood Cell | 0 |
| Red Blood Cell | 0 |
| Droplet | 0 |
| Fungal Candida | 0 |
| Bleed | 0 |
| O. Fragilis | 0 |
| E. Nuclei | 0 |
| E. Fragment | 0 |
| E. Trophozoite | 0 |
| E. Cell | 0 |
| Oocyst | 0 |
| Cybe | 0 |
| Isosporidia | 0 |
| Cell Group JB | 0 |

Classification Summary:

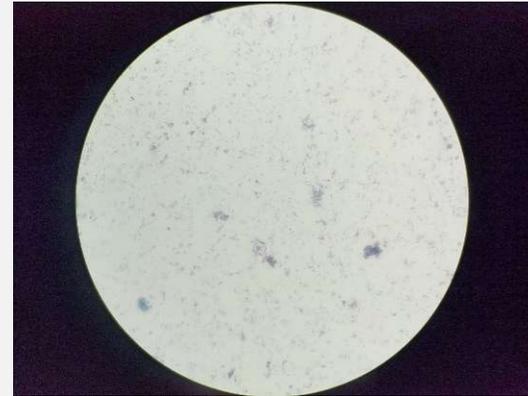
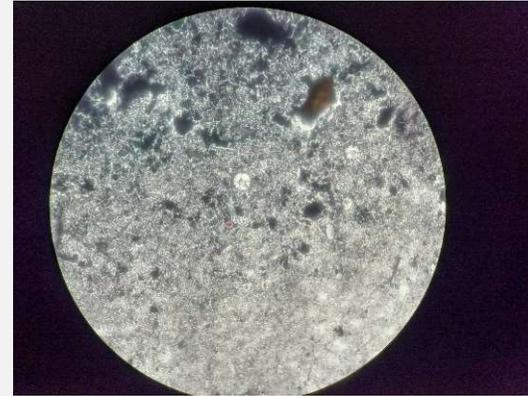
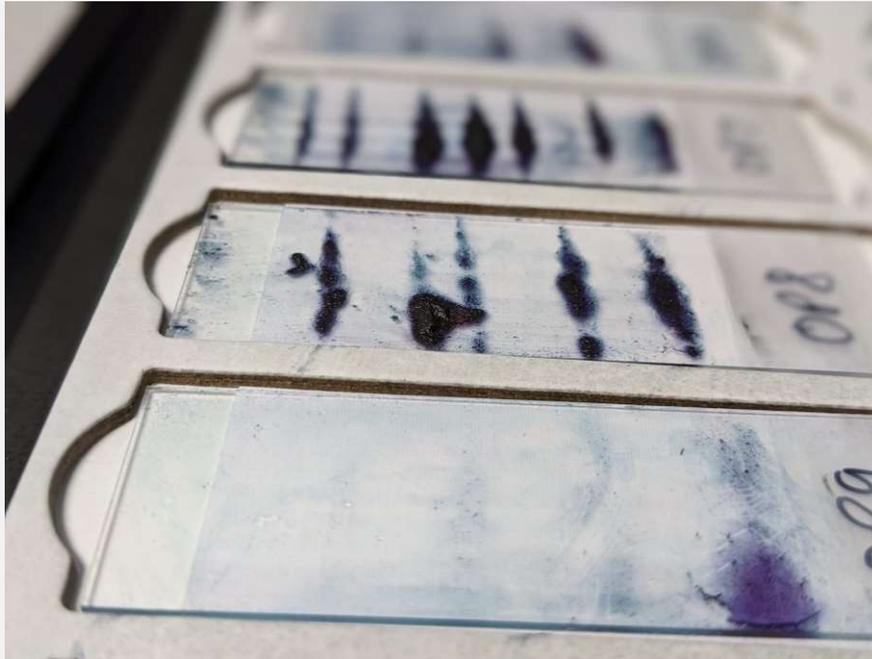
- Artifacts: 2748
- Other Negatives: 150
- Blasto: 1623
- Positive: 137
- Total: 8665

O&P
Trichrome
DNN Analysis

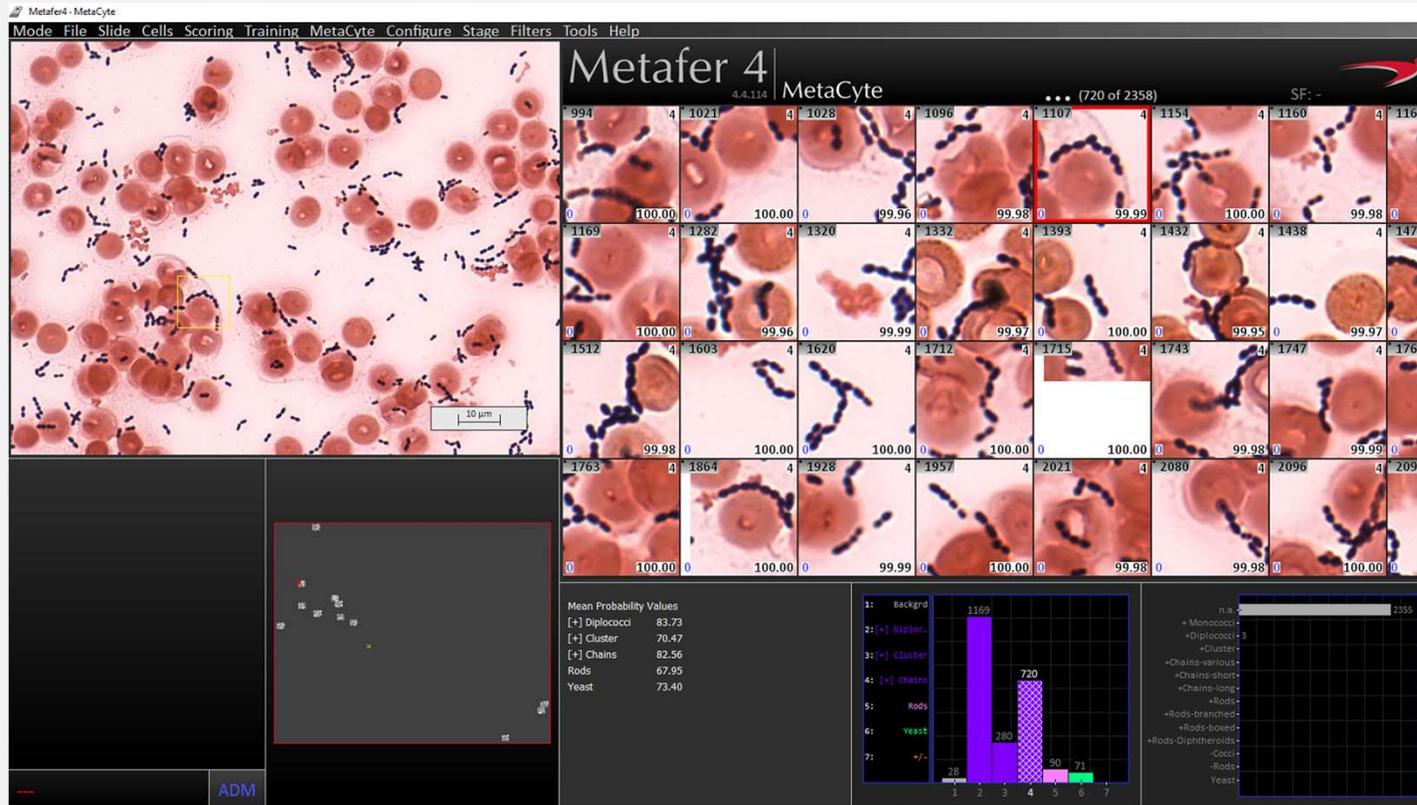
ADM

Setup
Search
Gallery
Field Review 2
Ikaros
Exit

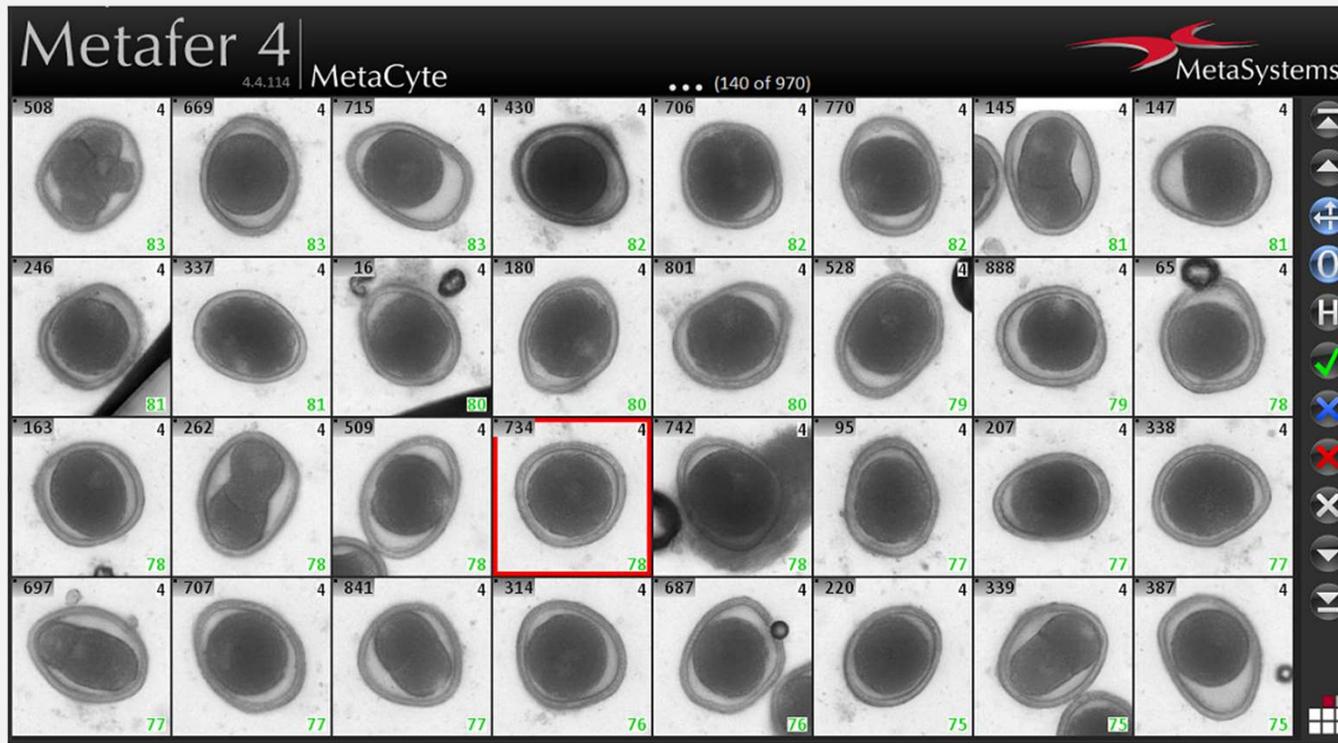
OVA & PARASITE



GRAM STAIN



OVA & PARASITE



FUTURE OUTLOOK

- Sample collection
- Accessioning
- Sample preparation
- Processing
- Data acquisition
- Analysis/identification
- Resulting/reporting

