REAL-TIME DATA PROCESSING WITH DEEP LEARNING FOR ADVANCED MICROSCOPY

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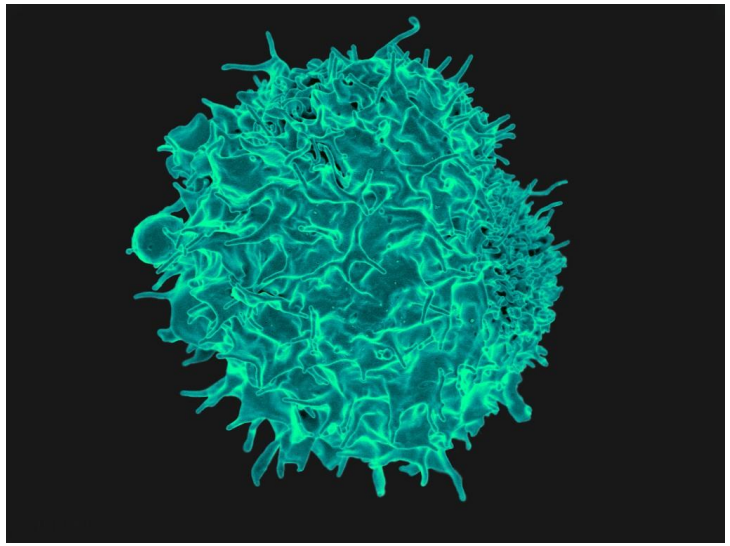
Imaging in biology

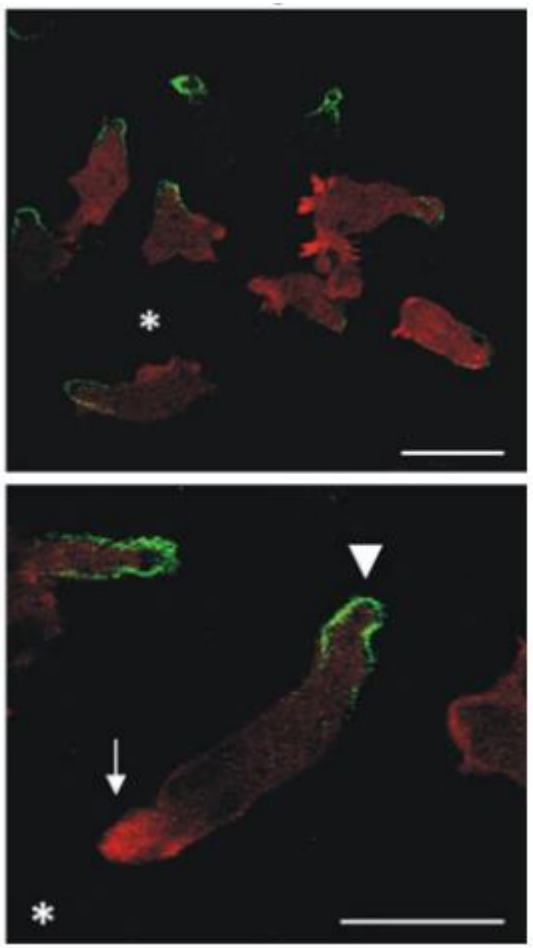
Most advances in biology are directly connected to microscopy

- Discovery by Hans and Zacharias Jannsen (1590)
- 1646 Athanasius Kircher: blood cells
- 1653 Petrus Borellus: use of microscope in medicine
- 1665-83 Hooke and van Leeuwenhoek: mocroorganisms
- 1882 Koch: tuberculosis and cholera bacilli
- 2012 electron microscope image of DNA



Nobel prices in chemistry in 2008, 2014, 2017 are related to microscopy

Microscopy in cell biology



Dual-color imaging of chemotaxing cells expressing GFP-PakA (green) and mRFP-LimEΔ (red). Cells were exposed to a gradient of cAMP released from a micropipette asterisk). Bars are 10 µm

From Müller-Taubenberger A., Ishikawa-Ankerhold H.C. (2013) Fluorescent Reporters and Methods to Analyze Fluorescent Signals. In: Eichinger L., Rivero F. (eds) Dictyostelium discoideum Protocols. Methods in Molecular Biology (Methods and Protocols), vol 983. Humana Press, Totowa, NJ

First microscope

Zeiss AxioObserver.Z1

Microscopy in cell biology: modern demands

For the advance of biology we require

- Study of rarely occurring events
- Screening large amount of samples
- Cell motility studies
- Control systems (Drugs administration, light activation)
- Reduction of the data size to be stored

Feedback Microscopy is a key to advance bioimaging

-> Real-time reliable processing is required

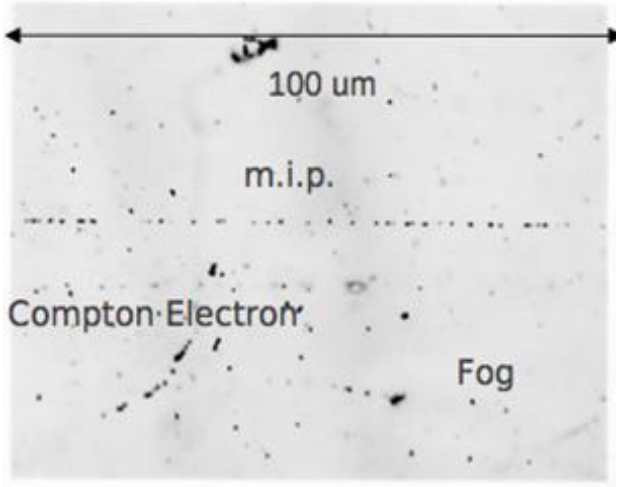
- Automated analysis tools don’t give satisfactory results
- Not enough performance for high-throughput microscopy

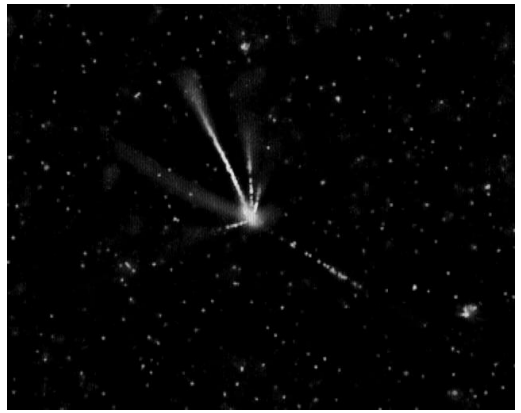
First microscope

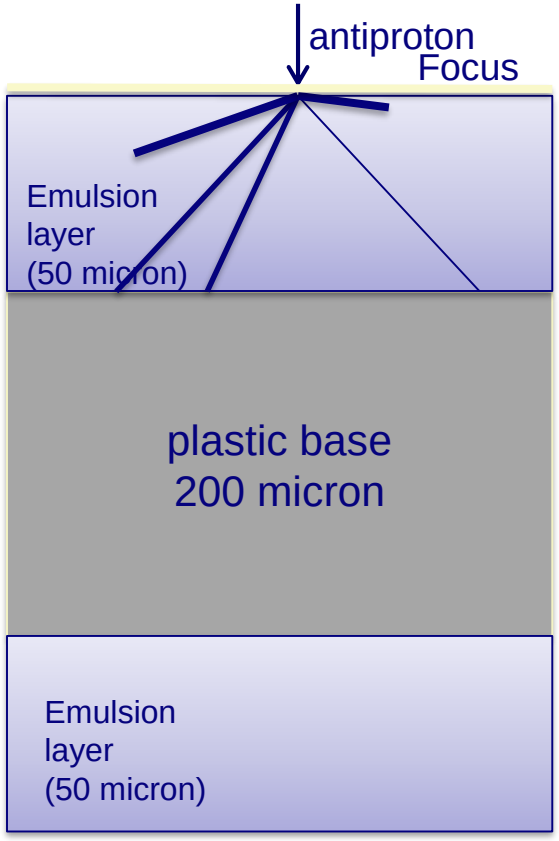
Zeiss AxioObserver.Z1

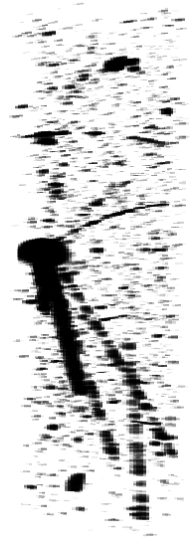
Microscopy for particle physics

- Nuclear photo-emulsion – high precision particle detector.
- Automated acquisition and reconstruction is immediate need.
- Back in 70's initiated development of automated real-time processing.
- It followed state-of-the-art electronics and computing technologies.





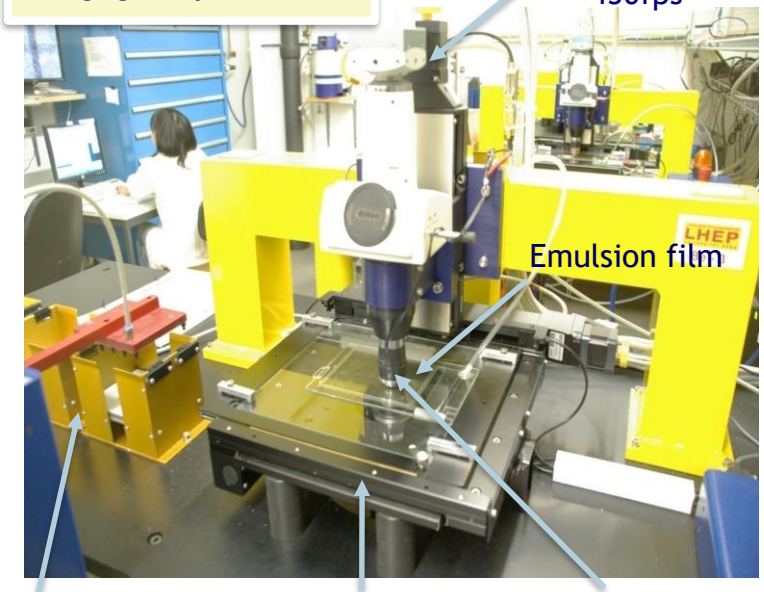


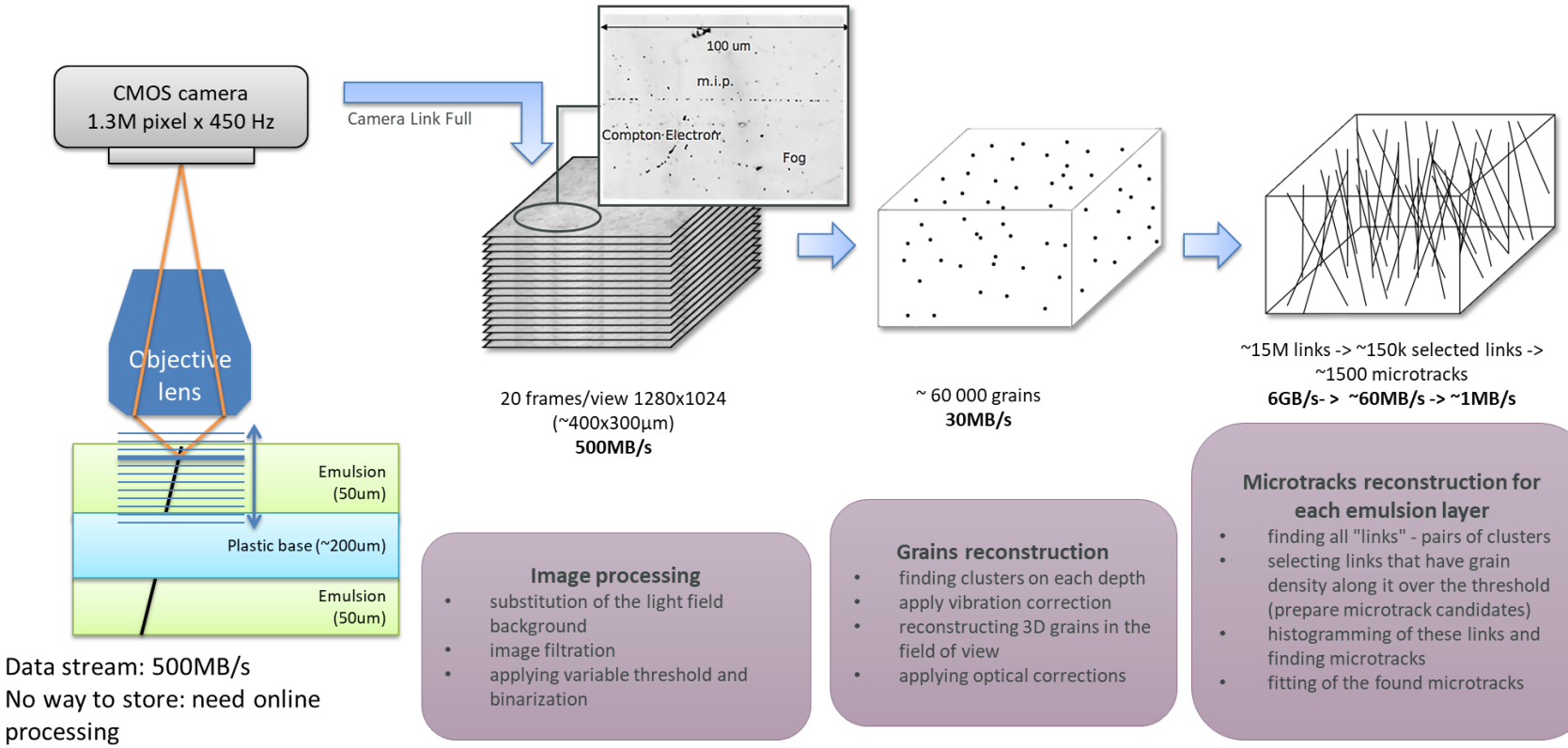


150 μm x 120 μm x 50 μm

Scanning system for emulsion detector readout

Readout speed
~20 cm² /h





Data stream: 500MB/s
No way to store: need online processing

Image processing
- substitution of the light field background
- image filtration
- applying variable threshold and binarization

Grains reconstruction
- finding clusters on each depth
- apply vibration correction
- reconstructing 3D grains in the field of view
- applying optical corrections

Microtracks reconstruction for each emulsion layer
- finding all "links" - pairs of clusters
- selecting links that have grain density along it over the threshold (prepare microtrack candidates)
- histogramming of these links and finding microtracks
- fitting of the found microtracks

6 automatic scanning stations in LHEP, currently upgrading
with new optics and 4MPix camera for 80cm²/h readout

Peak data rate in processing ~ 24Gb/s

A. Alexandrov, A. Buonaura, L. Consiglio, N. D'Ambrosio, G. De Lellis, A. Di Crescenzo, G. Galati, A. Lauria, M.C. Montesi, V. Tioukov, A new generation scanning system for the high-speed analysis of nuclear emulsions, J.INST, 11, 2016

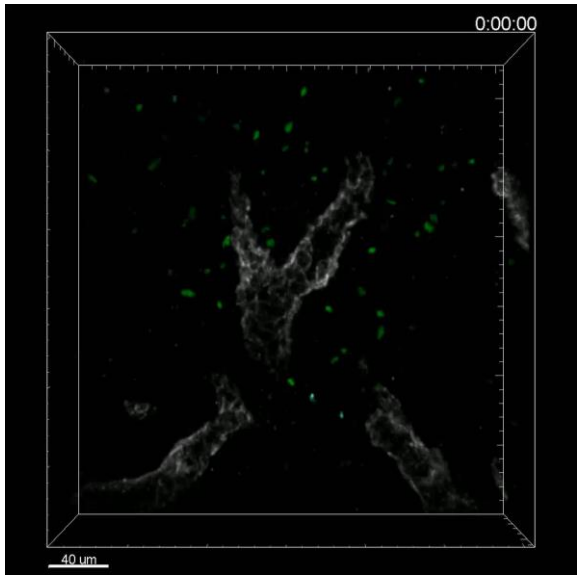
M.Vladymyrov, GPU-Accelerated Processing for Next Generation Nuclear Emulsion Scanning Systems GTC 2014, ID P4262

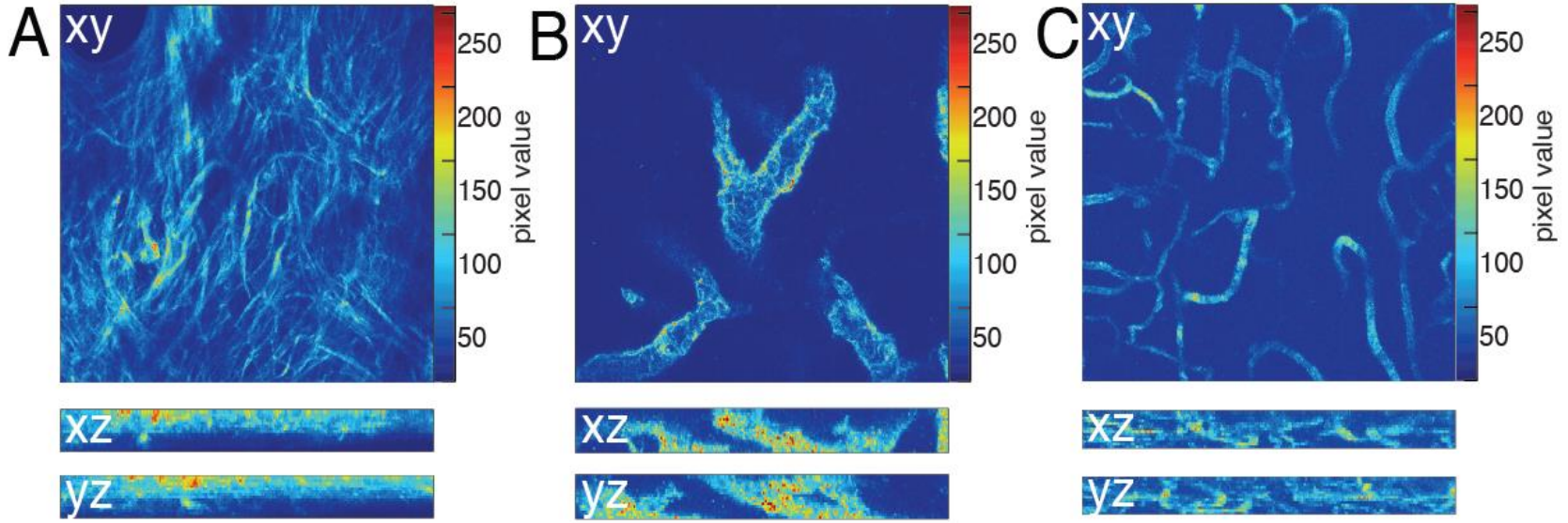
Application to biological imaging: Real-time offset correction

G-Track interdisciplinary project : LHEP and Theodor Kocher Institute, University of Bern: expanding live imaging in immunology using high-throughput analysis tools.

POSTER ID 23050 @GTC EU 2017

- Tissue drift is a big problem for long-term intravital acquisitions.
- Offset correction must be performed between acquisitions of consecutive time-frames.
- We developed a system performing correction using fine pattern matching in 3D using immotile anatomical landmarks
- Offset is then found using Pearson correlation coefficient separately in 3 projections. GPU implementation: 16ms
- Allows real-time tissue offset correction during data acquisition





Maximal intensity projections. SHG collagen layer (**A**), HEV in the LN (**B**), blood vessels marked with a fluorescent serum marker (**C**)

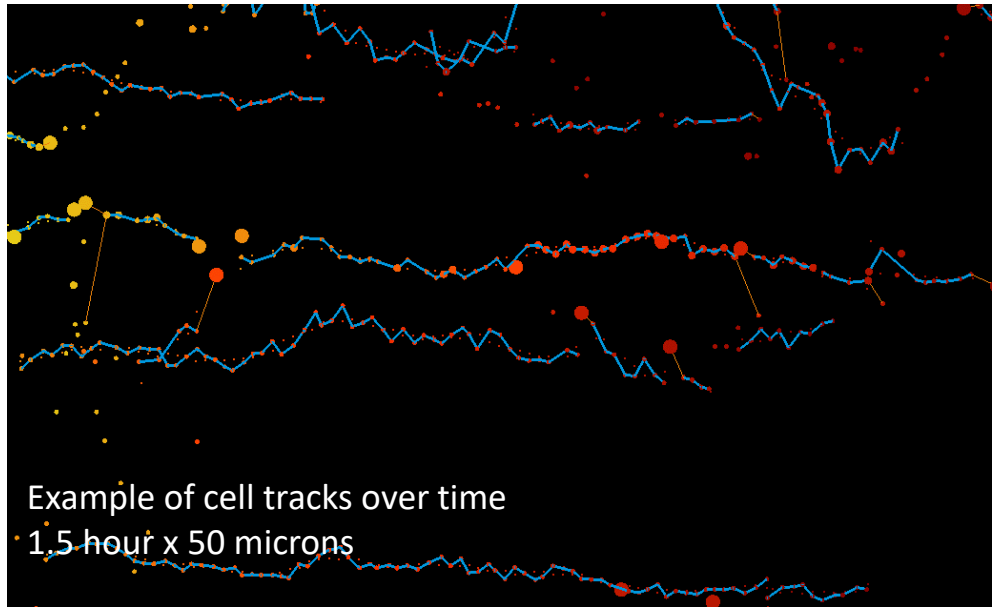
Vladymyrov M, Abe J, Moalli F, Stein JV, Ariga A. Real-time tissue offset correction system for intravital multiphoton microscopy. J Immunol Methods. 2016 Nov;438:35-41. doi: 10.1016/j.jim.2016.08.004.

Application to biological imaging: Real-time cell tracking

G-Track interdisciplinary project : LHEP and Theodor Kocher Institute, University of Bern: expanding live imaging in immunology using high-throughput analysis tools.

- Cell recognition: using GPU (15ms/timeframe)
- Cell tracking:
  - features not only coordinate-based cells linking but takes into account other cell parameters
  - Allows efficient cell to track assignment in case of 2 cells coming close using multi-variate likelihood analysis
  - Computational time < 5ms/timeframe

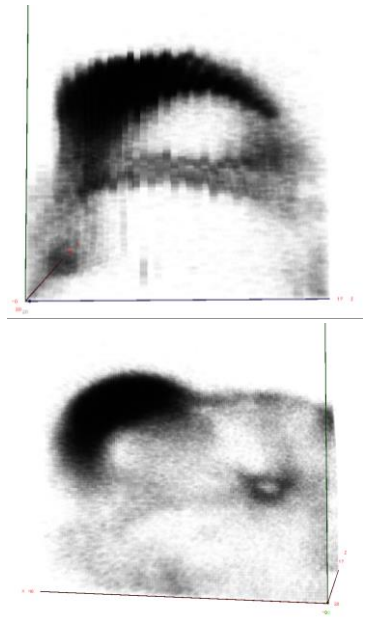
- Currently integrating in scanning pipeline for single cell following over time

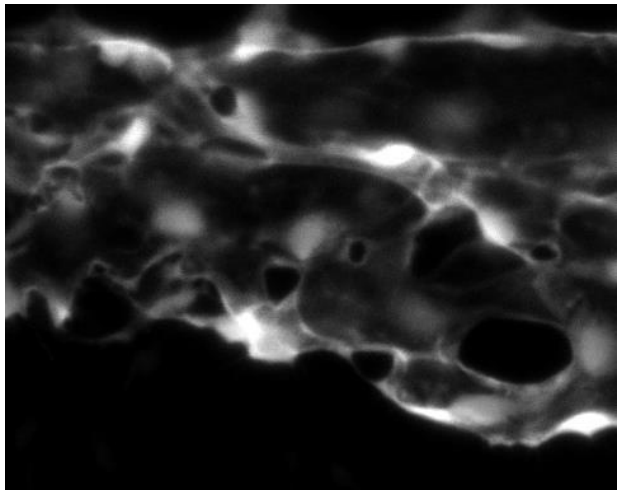


Advanced samples

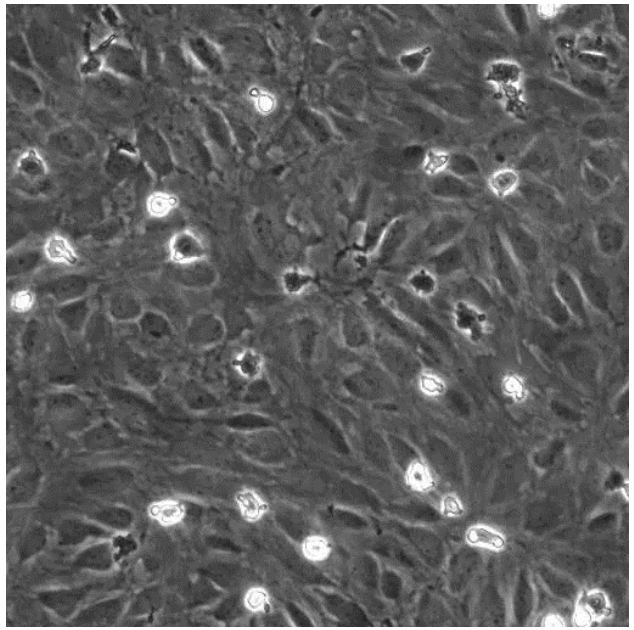
Sometimes this is not enough:

- Noise
- Dense cultures
- Search for complicated shapes



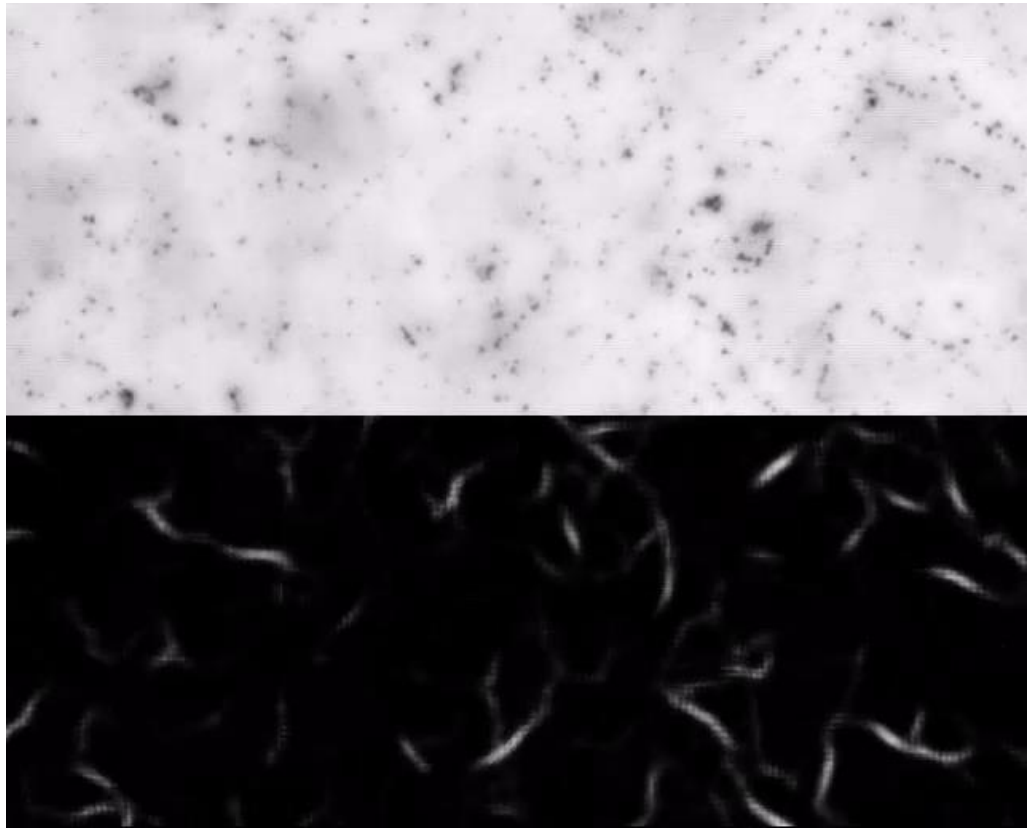


Intraluminal pillars in zebrafish embryos

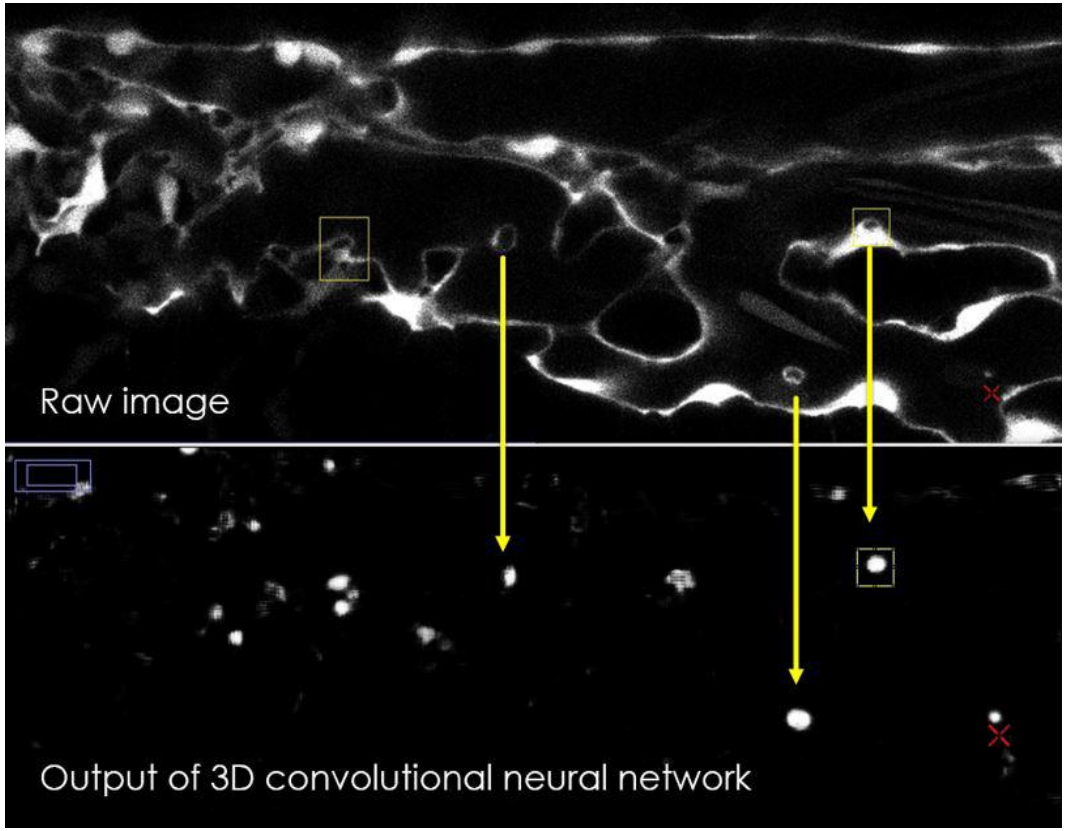


T-cells on microvascular endothelial cells. Modelling Blood Brain Barrier in a flow chamber.

Advanced samples: DL



Low energy electrons' tracks in emulsion detector



Intraluminal pillars in zebra fish embryo

Usual DNN inference performance is not enough: ~0.1-10 MB/s
High throughput imaging: 50-200MB/s (SPIM/SDCM)

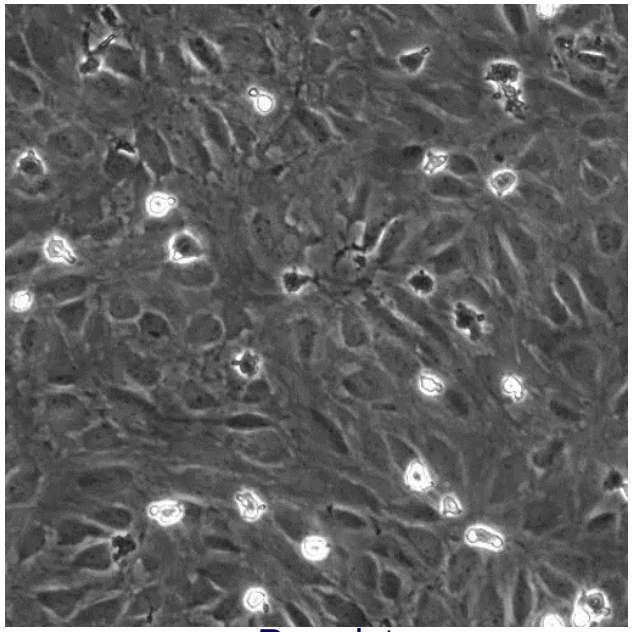
Network should be trained for a particular dataset (There are hundreds of different mics and thousands of prep configuration out there!)

Cells in dense tissue

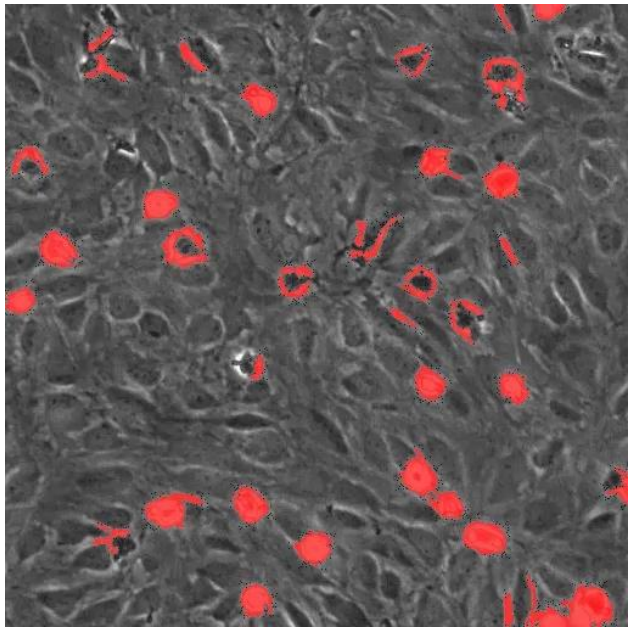
Problems:

- segmentation of cells in dense tissue
- cell clumps.
- noisy images
- on substrate of similar cells

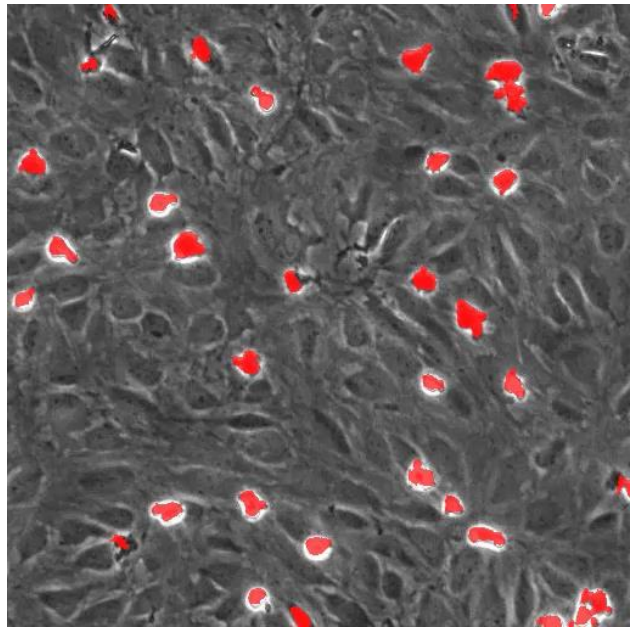
T-cells on microvascular endothelial cells.
Modelling Blood Brain Barrier in a flow chamber.



Raw data



Thresholding + segmentation



DNN, 2D+T
6L, ~80k params

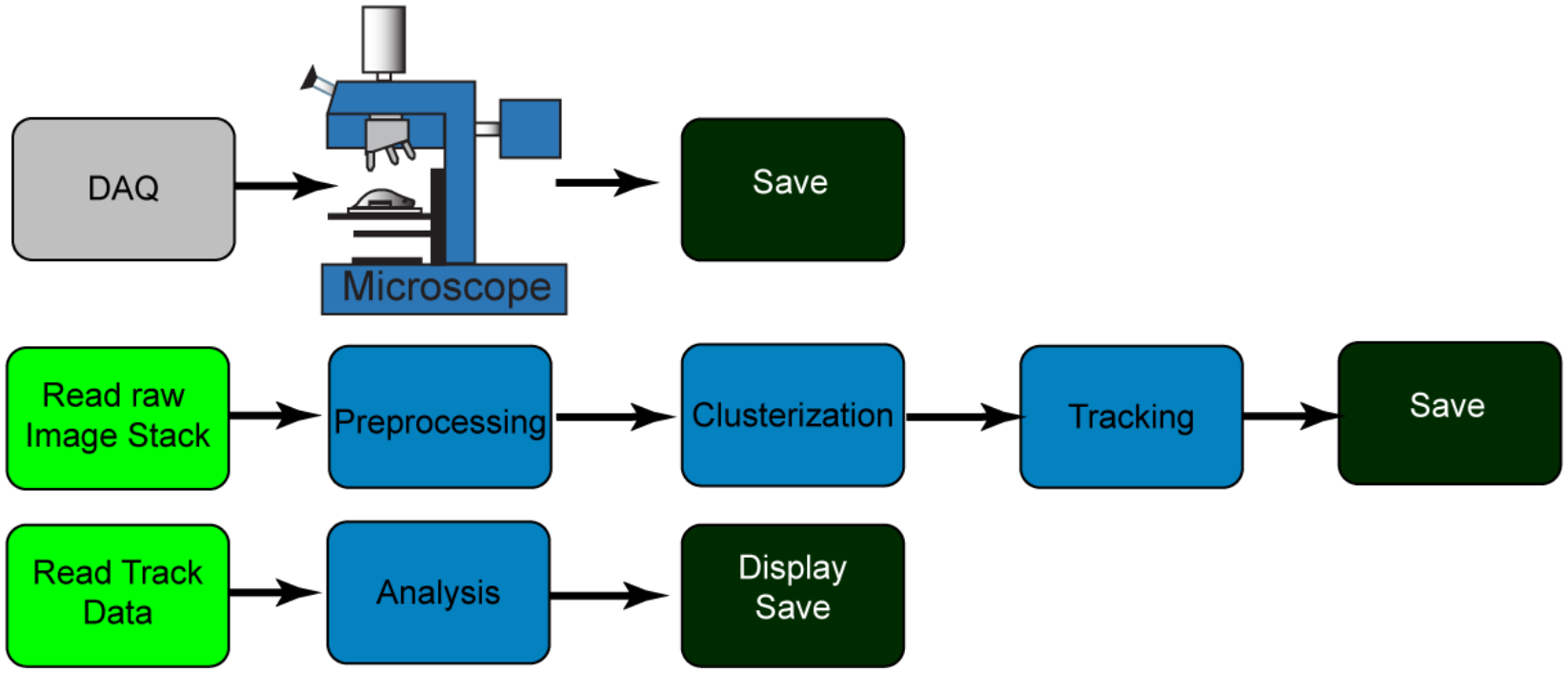
DeepMic: platform for feedback microscopy with intelligent data processing

DeepMic will combine efficient object detection using DL with real-time processing, providing:

- Accurate segmentation (developmental, cell biology, immunology, *in-vivo* and *in-vitro*)
- Real-time processing (automated high-throughput screening of large amount of samples)
- Compatibility with most microscope systems (Zeiss, LVBT, Leica / uManager)
- Visualization of cells and parameters during acquisition
- Feedback loop

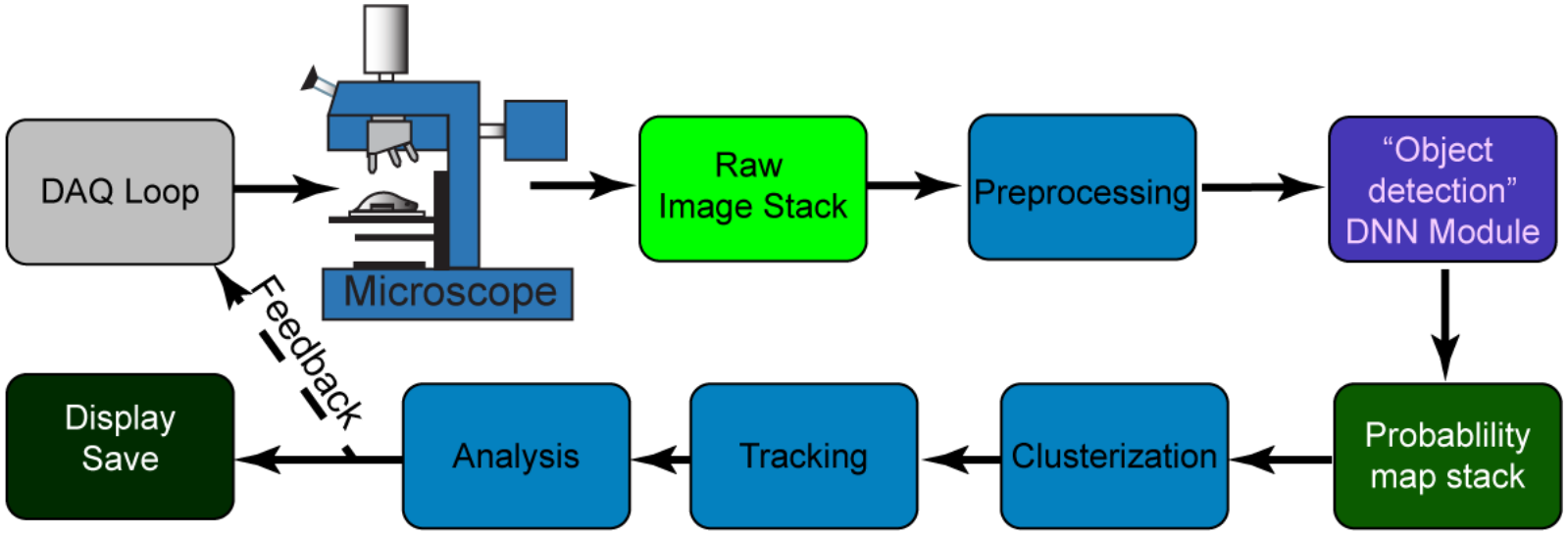
Usual pipeline

In most applications image acquisition and processing are separated



Real-time pipeline with DNN segmentation

0.1 – 10 MB/s microscopes (confocal, 2PM)

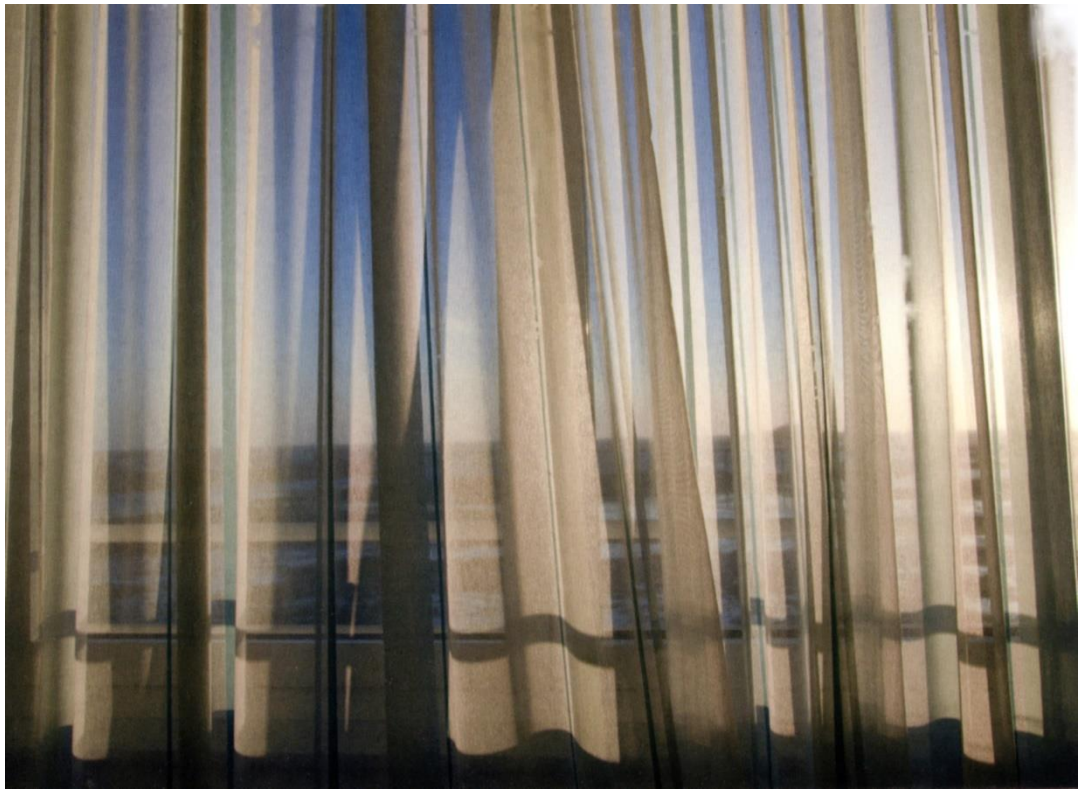


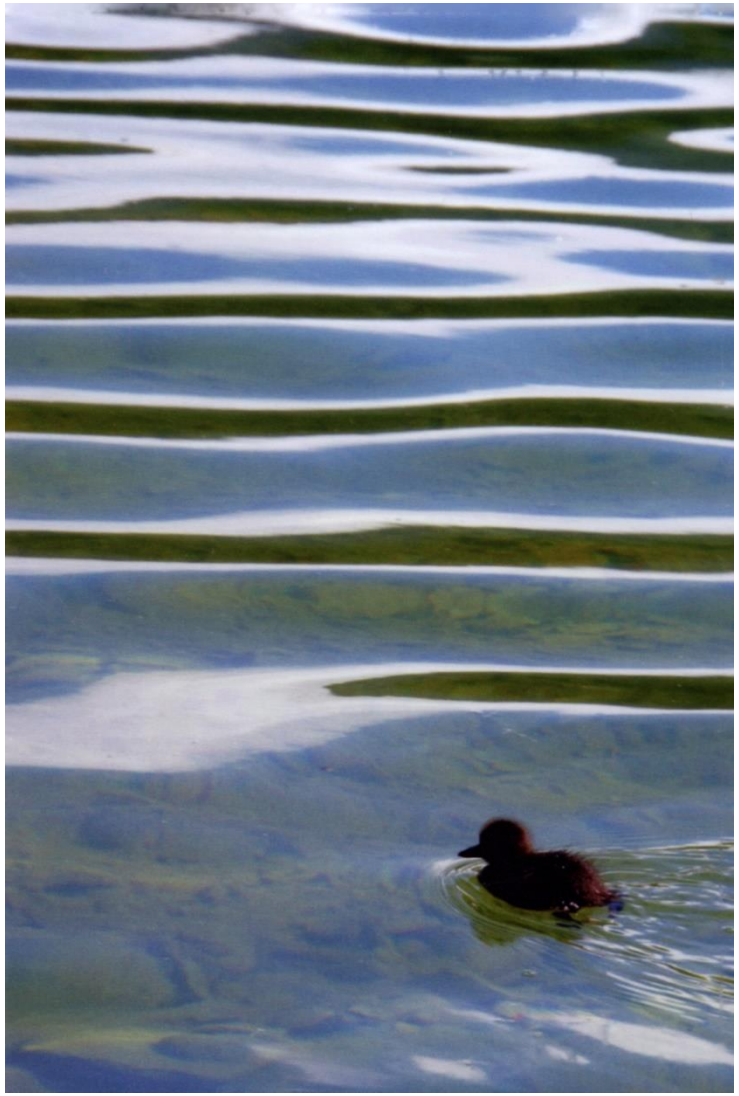
Importance of context for proper detection

It’s clear that in many cases human surpasses AI in image recognition tasks.

- Human knows full (much bigger) context
- Knowledge from different fields that might be required
- Rough idea what is important and what is not

Importance of context for proper detection





© Martina Issler, Zurich.
^ Ein Morgen am Meer. 2016
> Von Welle zu Welle. 2010

DeepMic

Object segmentation – way more hard then locating objects.

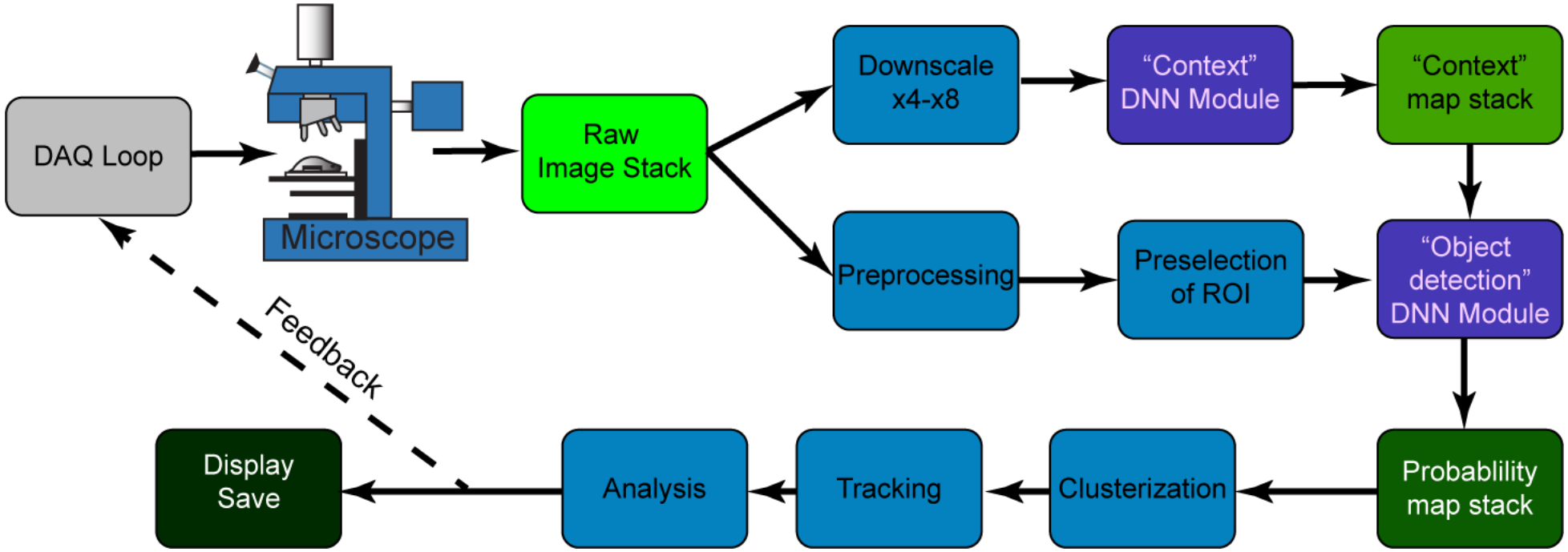
Objects of interest – often few % of area.

Context – may be 100%.

Our approach: modular structure

Real-time pipeline for High Throughput microscopy

30 – 200 MB/s microscopes (SPIM, SDCM)

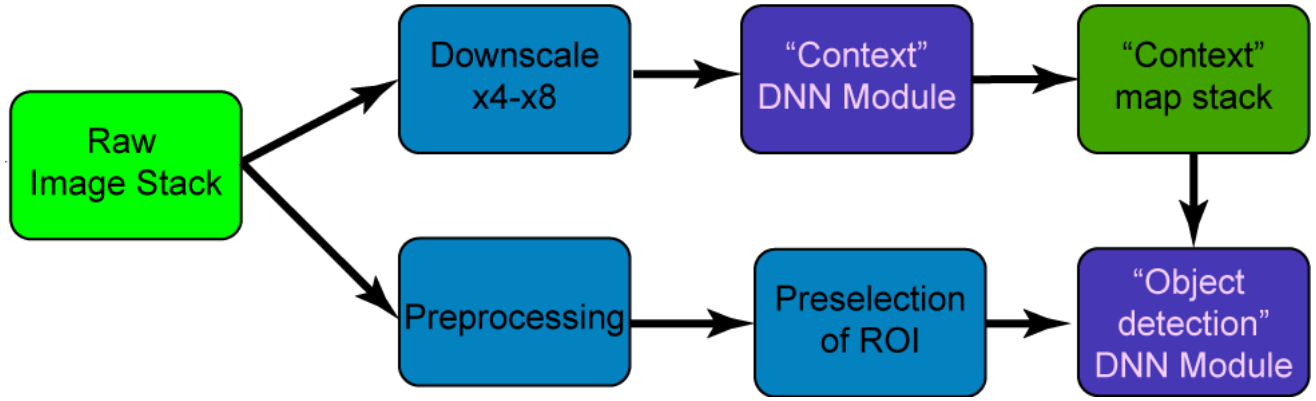


2-part networks Context and detection -> speed + efficiency

Real-time pipeline for High Throughput microscopy

Preselection – usually 1-5% of the region
Context module: 150k params, 6 layers
Input: 512 x 512 x 16 voxels
Output: 32 feature volumes 456 x 456 x 12

Performance: < 20ms / volume,
>200MVox/s – enough for High throughput microscopy



Conclusion and Outlook

- Feedback Microscopy is crucial for advance of biological research and medicine
- Real-time processing is the core of Feedback Microscopy
- Real-time tomographic image processing is well established in the field of particle physics and successfully applied in immunology

- Deep Learning is required for successful analysis of many biological samples
- Module architecture can allow
  - Real-time processing
  - Faster adaptation for new configurations

- Combination of “classical” approached and DL can provide required performance
- We are working on the full pipeline

Thank you!

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CHECK OUT OUR POSTER:
ID 23050

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