Cell Death: Many Ways to Die.

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Brief History of Cell Death Research

1842 - Karl Vogt noticed dying cells in toads (formation of vertebrae). The first scientific observation of regulated cell death (RCD).

1965 - Lockshin and Williams - specific cells die during the metamorphosis of the silkworm, this type of cell death is 'programmed' because these cells were destined to die according to a 'construction manual' for the insect.

1972 - Kerr *et al*. - specific type of cell death in human tissues in which the cells and nuclei became condensed and fragmented, and they called this cell death process 'apoptosis'. They proposed that apoptosis is crucial for regulating cell populations during tissue development and turnover.

Classification of cell death: recommendations of the Nomenclature Committee on Cell Death

G Kroemer ✉, W S El-Deiry, P Golstein, M E Peter, D Vaux, P Vandenabeele, B Zhivotovsky, M V Blagosklonny, W Malorni, R A Knight, M Piacentini, S Nagata & G Melino

Cell Death & Differentiation **12**, 1463–1467 (2005) | Cite this article

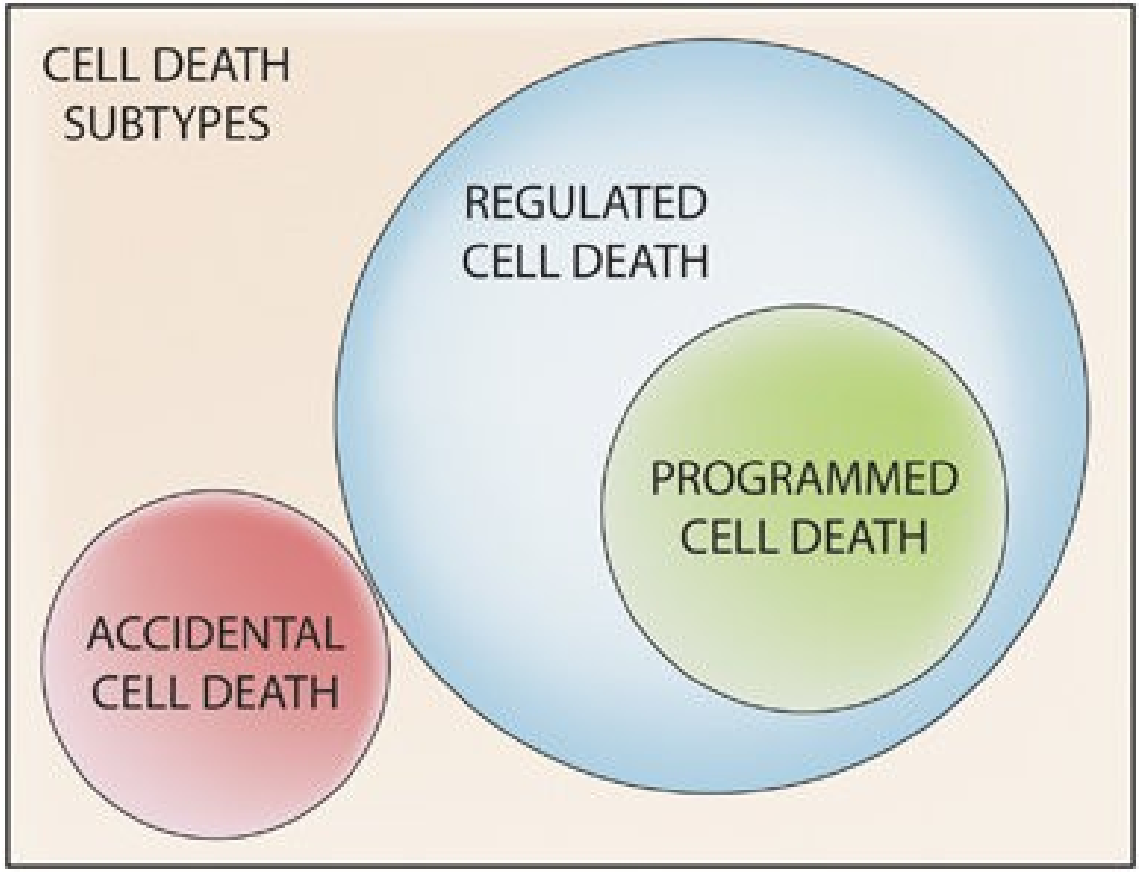
18k Accesses | **578** Citations | **9** Altmetric | Metrics

Lockshin, R. Programmed cell death 50 (and beyond). *Cell Death Differ* **23,** 10–17 (2016). https://doi.org/10.1038/cdd.2015.126

Tang, D., Kang, R., Berghe, T.V. *et al.* The molecular machinery of regulated cell death. *Cell Res* **29,** 347–364 (2019). https://doi.org/10.1038/s41422-019-0164-5

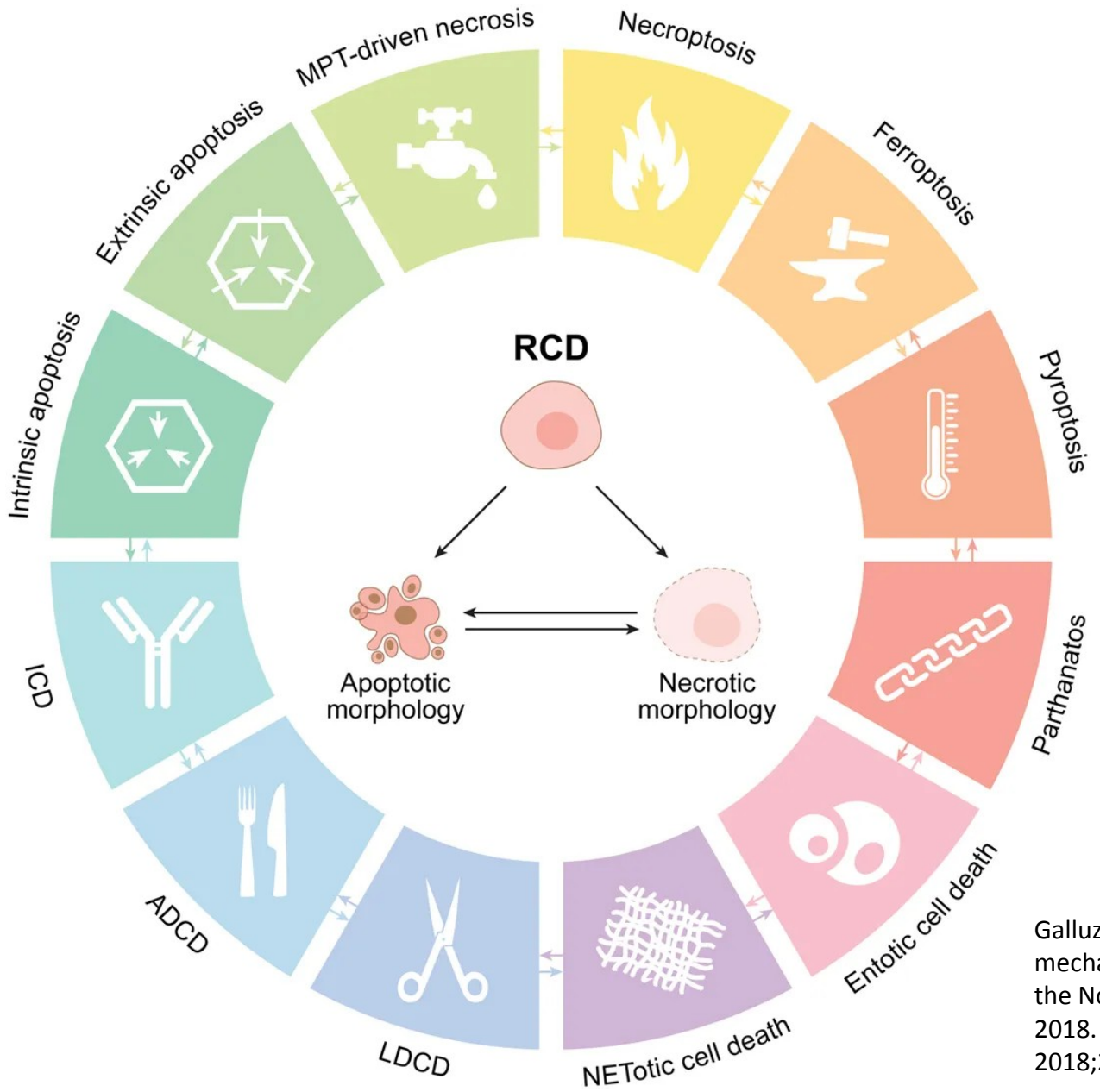
Accidental vs. Regulated Cell Death

- **Cell death plays a central role in all aspects of life**. It is involved in the development of multicellular organisms and tissue homeostasis where cell death depletes dispensable cells.
- Cells may die from **accidental cell death** (ACD) or **regulated cell death** (RCD).
- **ACD is a biologically uncontrolled process**, whereas **RCD involves tightly structured signaling cascades and molecularly defined effector mechanisms**.
- Cell death is critical for fighting off infections and is associated with multiple diseases that are caused by deregulated or dysfunctional cell death signaling.



Galluzzi, L., Bravo-San Pedro, J., Vitale, I. *et al.* Essential *versus* accessory aspects of cell death: recommendations of the NCCD 2015. *Cell Death Differ* **22,** 58–73 (2015). https://doi.org/10.1038/cdd.2014.137

Regulated Cell Death



Galluzzi L, Vitale I, Aaronson SA, et al. Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. Cell Death and Differentiation. 2018;25(3):486-541.

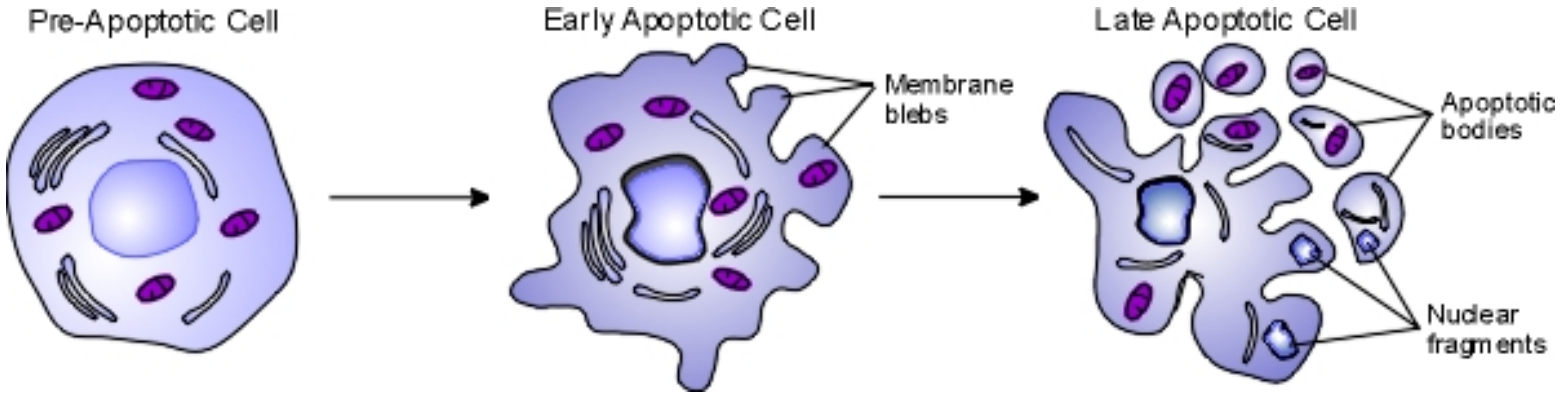
Apoptosis

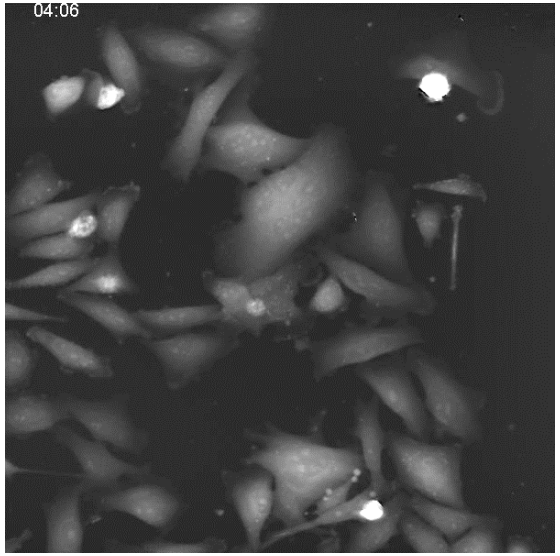
- Apoptosis is the first described form of programmed cell death, and it plays a critical role in tissue homeostasis.
- It contributes to cell turnover, the proper functioning of the immune system, and embryonic development.
- There are several **key characteristics of apoptosis**:

cellular, organelle, and DNA fragmentation and formation of apoptotic bodies

active, energy consuming process executed by a subset of cellular proteins

Even though, in general, this **process is immunological silent**, apoptosis has been shown to be involved in inflammatory pathologies as well.





Apoptosis

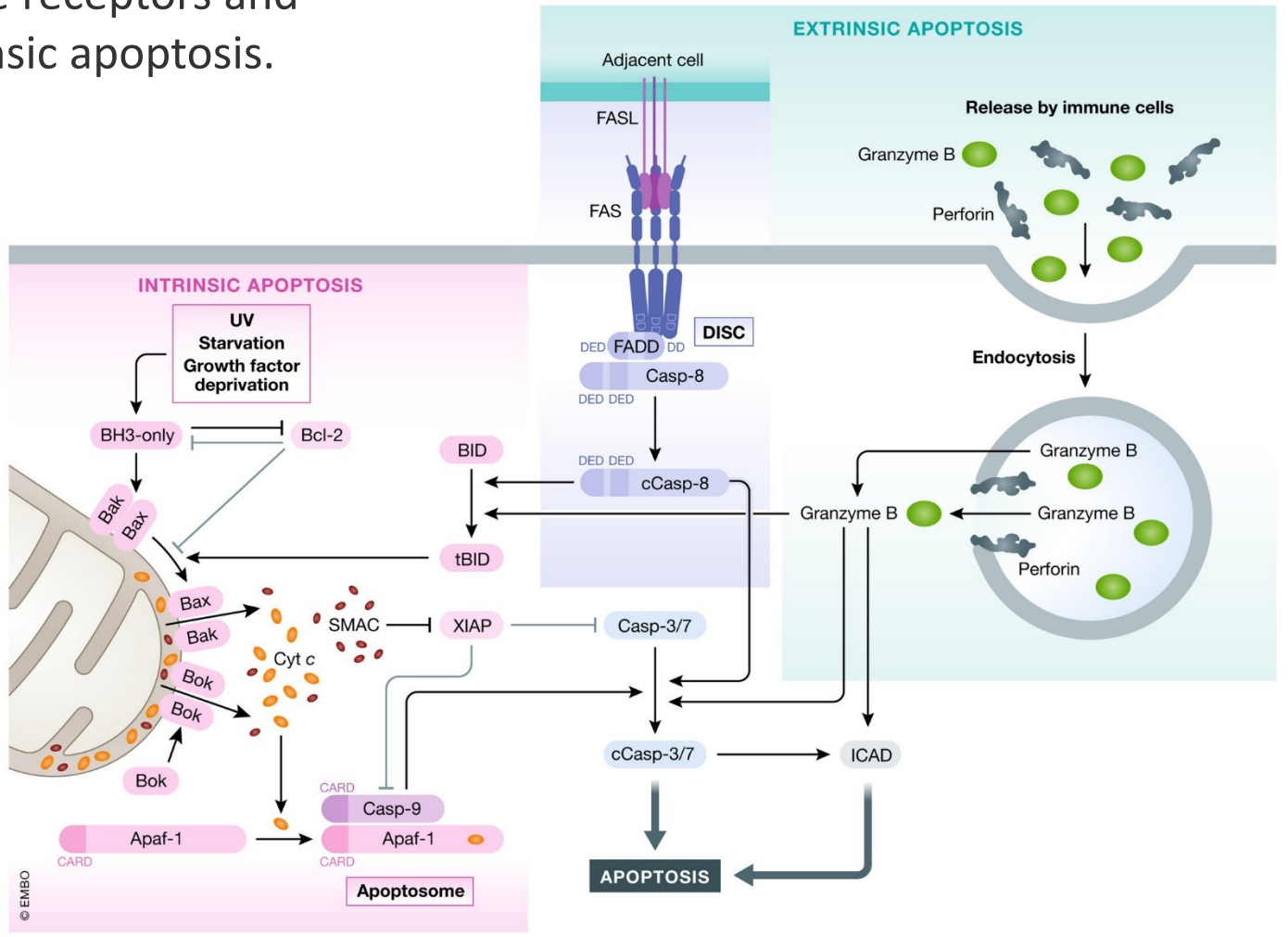
There are two (or 3) major pathways that mediate apoptosis: intrinsic and extrinsic pathways.

During **extrinsic apoptosis**, **TNF** (tumor necrosis factor) superfamily (TNFSF) can induce cell death by binding to their cell surface receptors and activating a deathly signaling cascade causing extrinsic apoptosis.

Intrinsic apoptosis is controlled by the equilibrium of the different **Bcl-2 (B-cell lymphoma 2) family members** which can be disrupted by various stimuli leading to cell death.

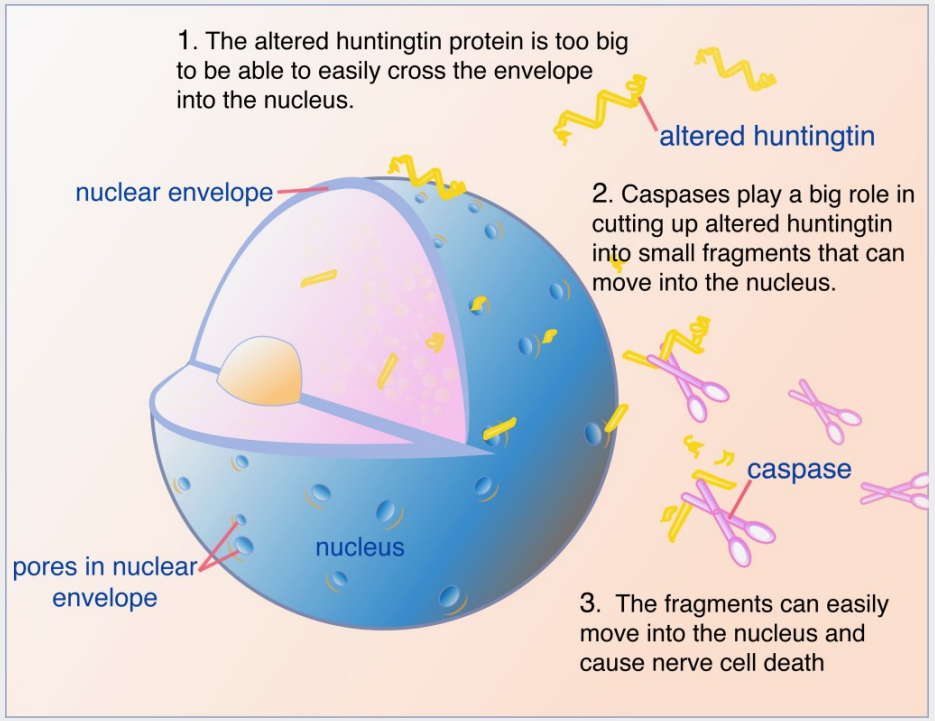
The third modality of apoptosis induction is **cell-based**. **Cytotoxic T cells** can engage cells that present non-self-antigens leading to cell death induction by **proteases called granzymes**.

All apoptotic pathways converge on the central proteases of this pathway: **caspases**, which are either playing a role in transmitting cell death stimulus **(initiator caspases)** or in the execution **(effector caspases).**



Caspases

- Caspases (cysteine-aspartate proteases) are proteolytic enzymes generally known for their role in controlling cell death and inflammation.
- Their role in cell death was described more than 20 years ago with the discovery of ced-3 as a trigger for cell death during the development of Caenorhabditis elegans.
- Caspases are involved in cell death by apoptosis, necroptosis and pyroptosis. Caspase function is not just about cell death.
- Non-apoptotic roles of caspases include proliferation, tumor suppression, differentiation, nervous system development and axon navigation, aging and angiogenesis.



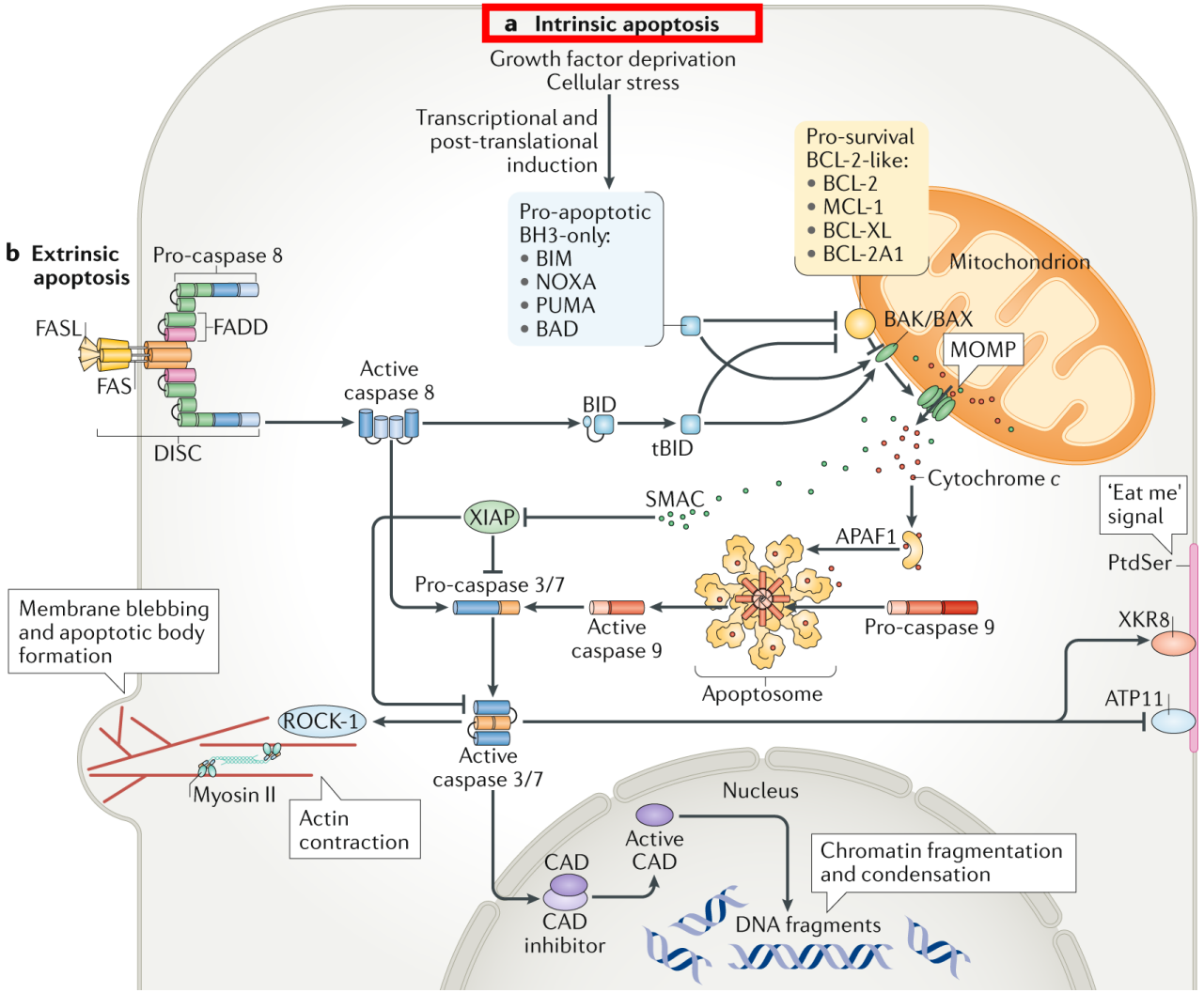
https://hopes.stanford.edu/caspase-6-inhibition/

Shalini S, Dorstyn L, Dawar S, Kumar S. Old, new and emerging functions of caspases. Cell Death & Differentiation. 2015;22(4):526-539.

Intrinsic Apoptosis

- Involves increases in the expression or activity of pro-apoptotic BH3-only proteins that bind with high affinity to members of the pro-survival BCL-2 protein family, which in healthy cells keep the effectors of apoptosis, BAX, and BAK, in inactive states.
- When all pro-survival BCL-2 proteins within a cell are functionally neutralized by BH3-only proteins, BAK and BAX are unleashed in order to oligomerize and assemble into structures that cause a breach of the outer mitochondrial membrane, thereby inducing mitochondrial outer membrane permeabilization (MOMP).
- MOMP causes the release of mitochondrial proteins.
- Cytochrome *c* binds to APAF-1 promoting formation of the apoptosome.
- Pro-forms of the initiator caspase 9 are recruited into the apoptosome, resulting in caspase 9 activation promoting the downstream proteolytic activation of the effector caspases 3 and 7.

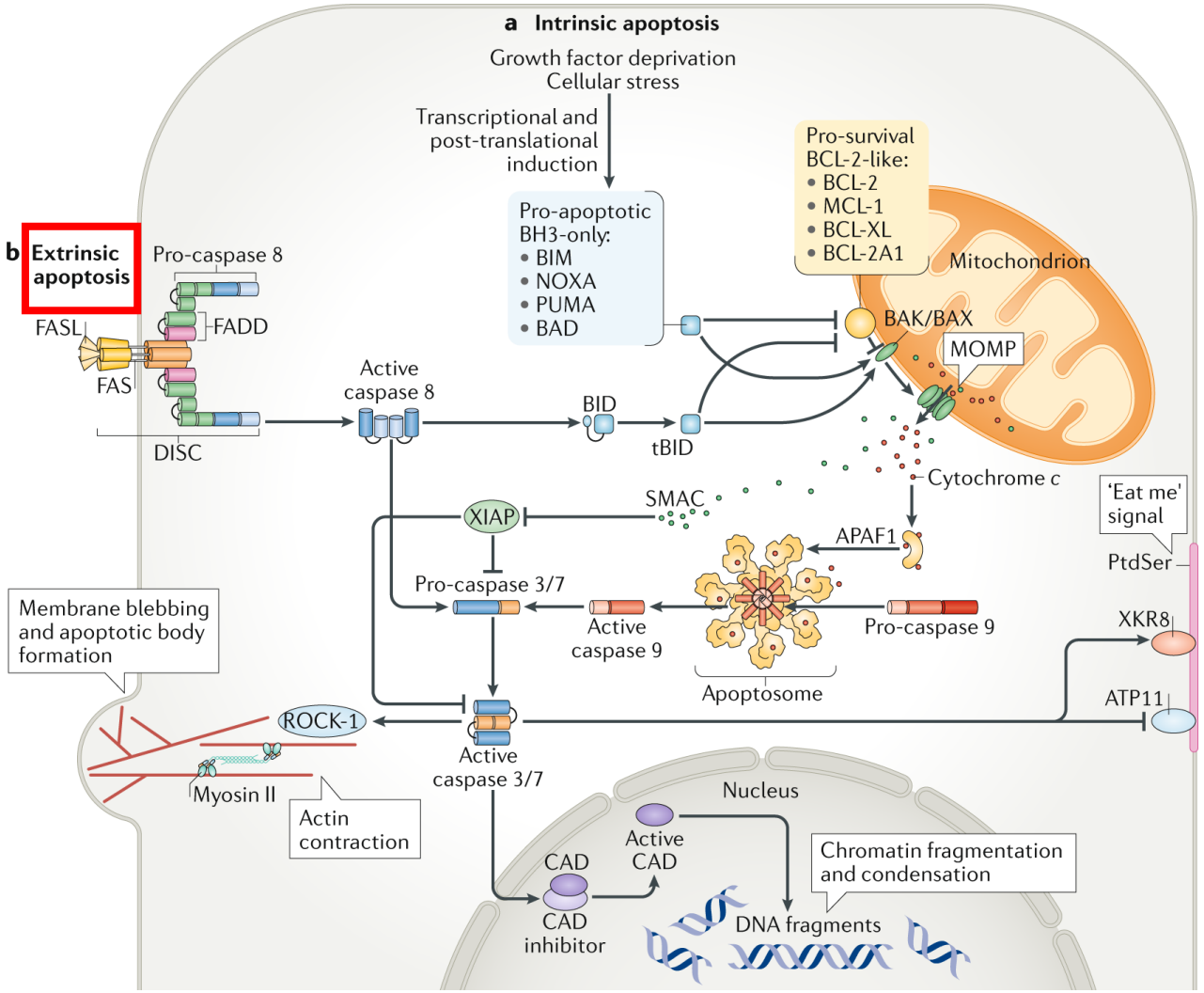
- Activation of caspase 3 and 7 cascade can be attenuated by XIAP, one of the inhibitor of apoptosis proteins (IAPs). MOMP also causes the release of SMAC (also known as DIABLO) and HTR2, which both can block XIAP and thereby prevent it from inhibiting caspases.



Bedoui, S., Herold, M.J. & Strasser, A. Emerging connectivity of programmed cell death pathways and its physiological implications. *Nat Rev Mol Cell Biol* **21,** 678–695 (2020). https://doi.org/10.1038/s41580-020-0270-8

Extrinsic Apoptosis

- Triggered by **TNF family ligand-receptor interactions**, most prominently by TNF family ligands: TNF, FasL, TRAIL, and TL1A.
- The receptor complexes either recruit FADD (Fas-associated protein with death domain) or TRADD (TNFRSF1A-associated via death domain) to the oligomerized complex.
- FasL binds to its transmembrane receptor Fas, which recruits FADD via death domain (DD) interactions.
- FADD contains a DD and also a death effector domain (DED), which allows the recruitment of caspase-8 forming the death inducing signaling complex—DISC.
- The proximity of multiple **caspase-8** molecules induces the **transactivation by proteolytic cleavage**.
- **Cleavage results** in the p18 and p10 fragments which **activate caspase-3 and caspase-7** (type I apoptosis).
- Insufficient activation of caspase-3 leads to type II apoptosis in which caspase-8 cleaves the BH3-only protein BID to generate its activated form: truncated BID (tBID).
- tBID stimulates intrinsic apoptotic pathway by directly binding to Bax/Bak inducing MOMP (type II apoptosis).
- The two pathways are cell line dependent, and their activation is differentially regulated by XIAP expression.



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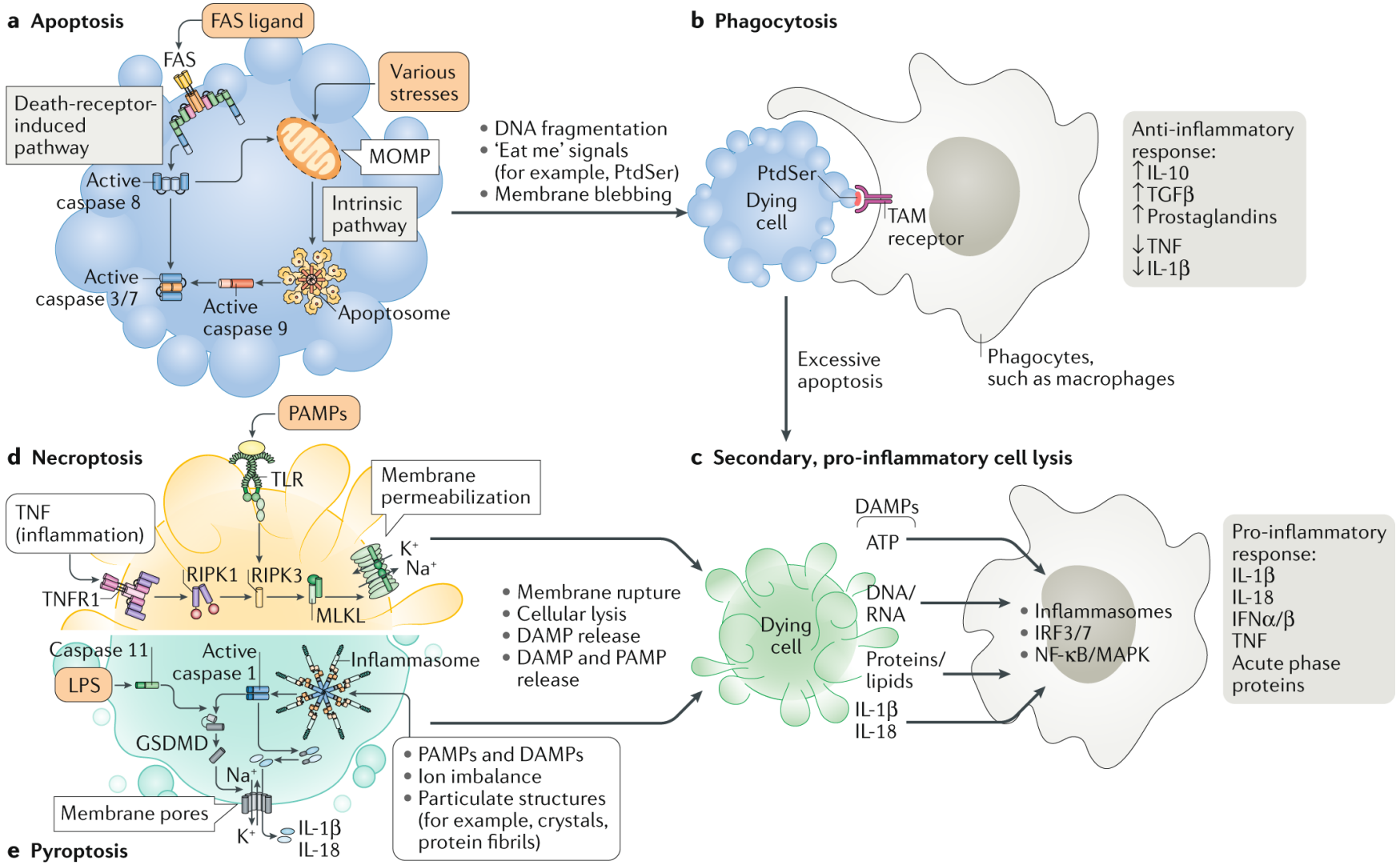
The EMBO Journal (2021) 40: e106700.

Regulated Necrosis vs Apoptosis - Lytic vs Non-lytic cell death

- **Non-lytic cell death, apoptosis (the integrity of plasma membrane is sustained).**
- **Plasma membrane rupture (PMR) is the final cataclysmic event in lytic cell death (regulated or accidental necrosis).**
- **PMR releases intracellular molecules known as damage-associated molecular patterns (DAMPs) that propagate the inflammatory response.**



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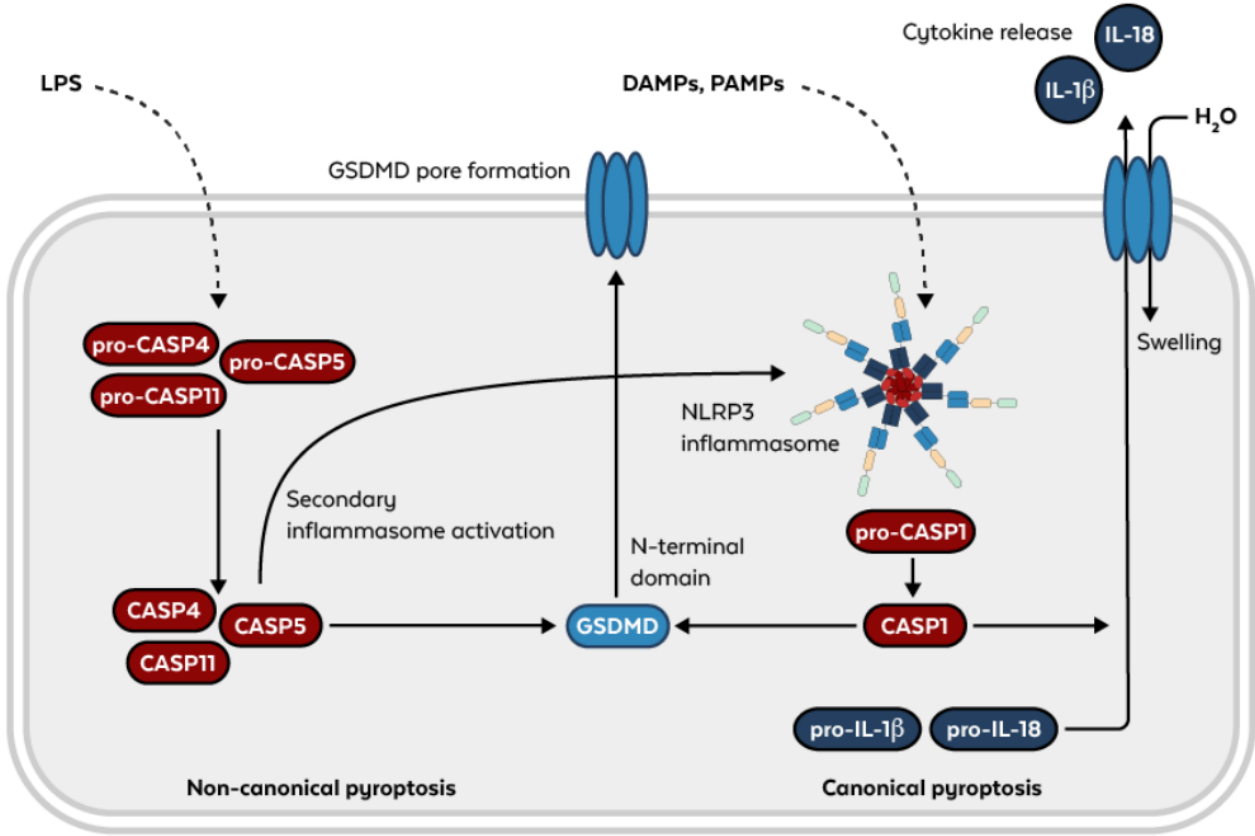
Pyroptosis

Pyroptosis is a potent inflammatory mode of lytic cell death triggered by diverse infectious and sterile insults. It is driven by the pore-forming fragment of gasdermin D (GSDMD) and releases two exemplar proteins:
pro-inflammatory cytokine IL-1β, and IL18, a standard marker of PMR and lytic cell death.

Two sequential steps for pyroptosis:

- initial formation of a small plasma membrane pore that causes the release of IL-1β and non-selective ionic fluxes
- subsequent PMR attributable to oncotic cell swelling with final PMR by NINJ1 protein.



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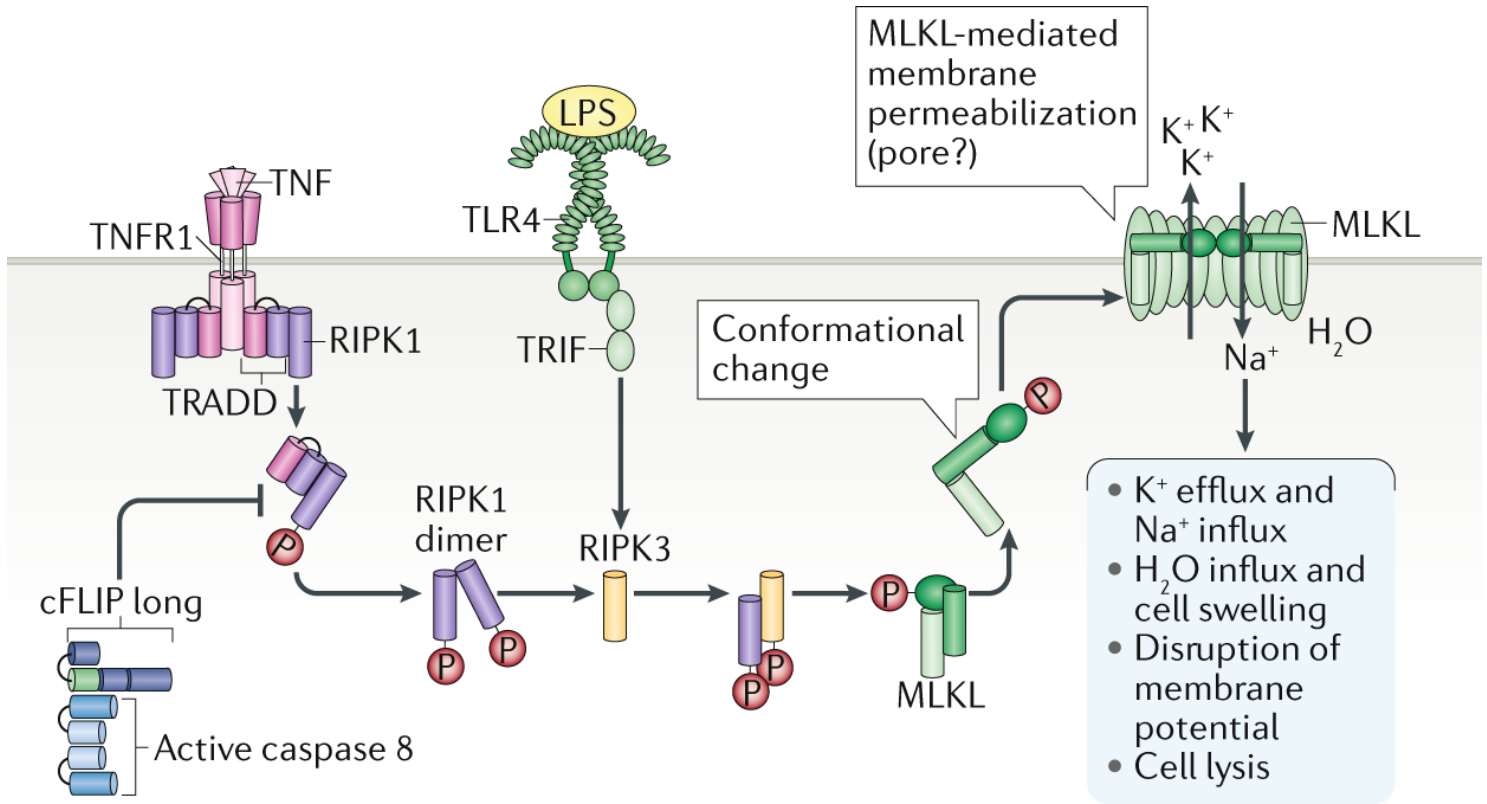
Necroptosis

Necroptosis is a pathway for genetically **programmed lytic cell death** that is thought to have a role in the killing of pathogen-infected cells and/or damaged cells during certain degenerative or inflammatory disorders.



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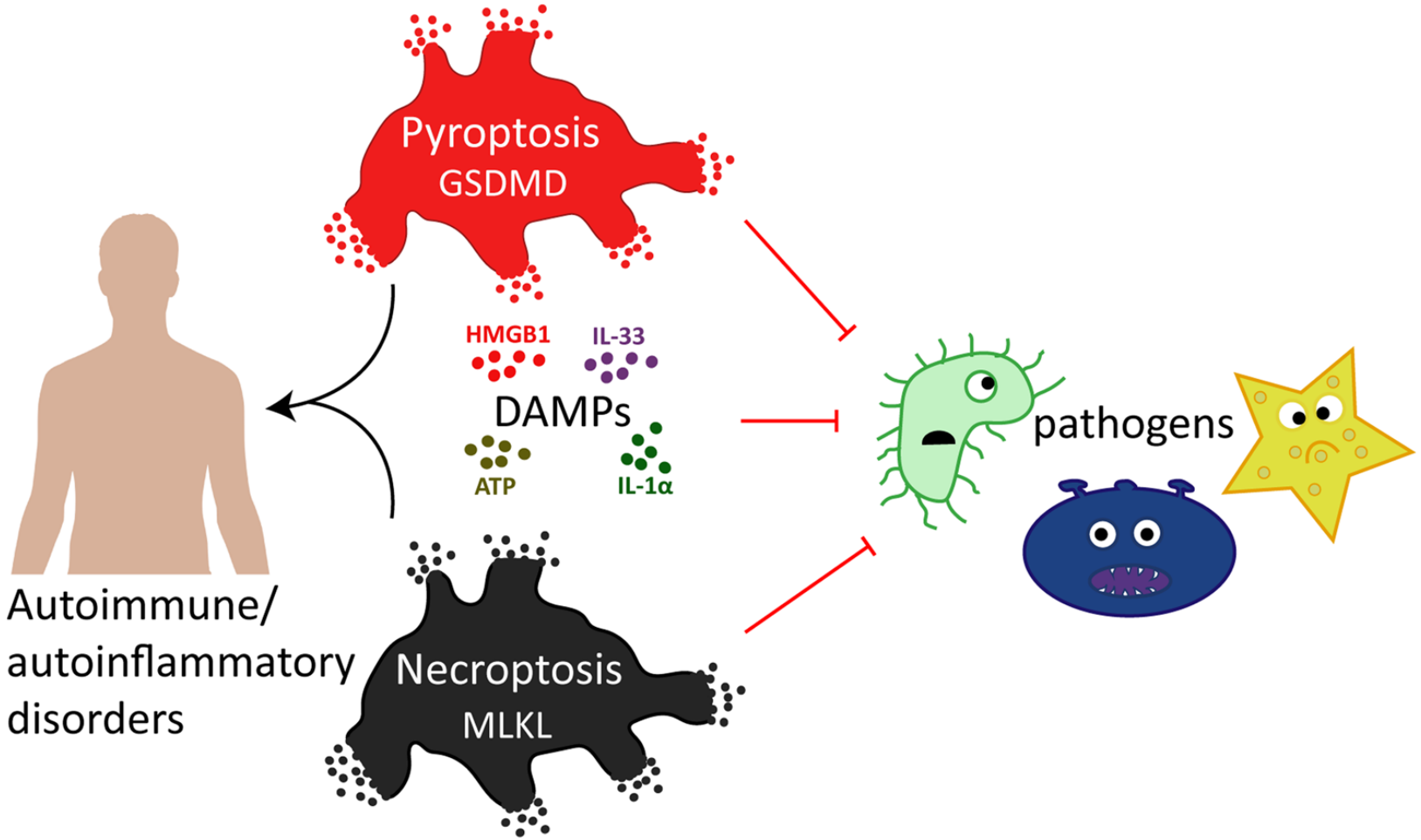
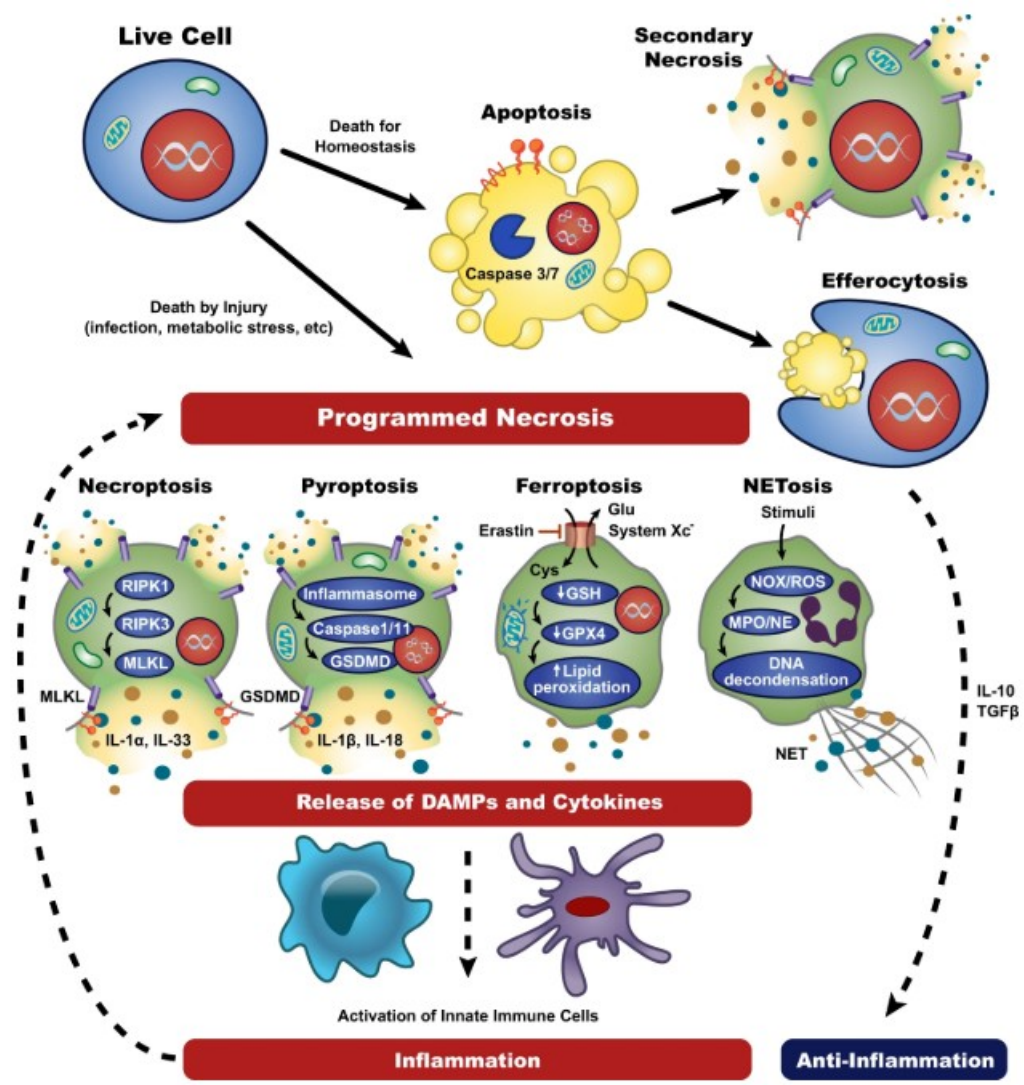
The role of cell death in host responses to infection.



Although necrosis and pyroptosis are important barriers against microbial pathogens, disruption of their regulation causes numerous autoimmune and inflammatory conditions leading to various diseases.

Regulated Cell Death



Necroptosis, pyroptosis, ferroptosis, and NETosis are types of programmed necrosis where lytic cell death is mediated by an activatable genetic program.

Accidental and programmed necrosis share morphological features: Swelling of the cell and permeabilization of the cell membrane associated with the release of potentially dangerous contents of the dying cell (DAMPs) - induction of inflammation.

Inflammation associated with necrosis is caused by inflammatory cytokines and DAMPs (cell molecules released into the environment with loss of membrane integrity) from cells subject to necrotic cell death.

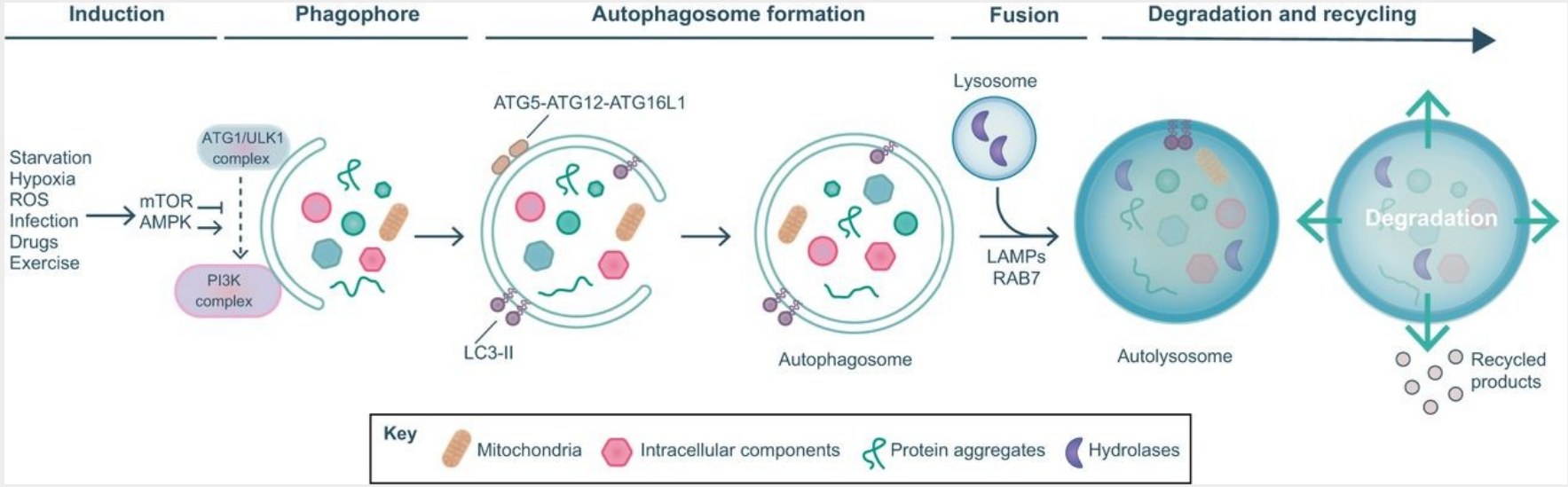
Defects in programmed necrosis and efferocytosis are associated with the development of inflammation and autoimmune diseases.

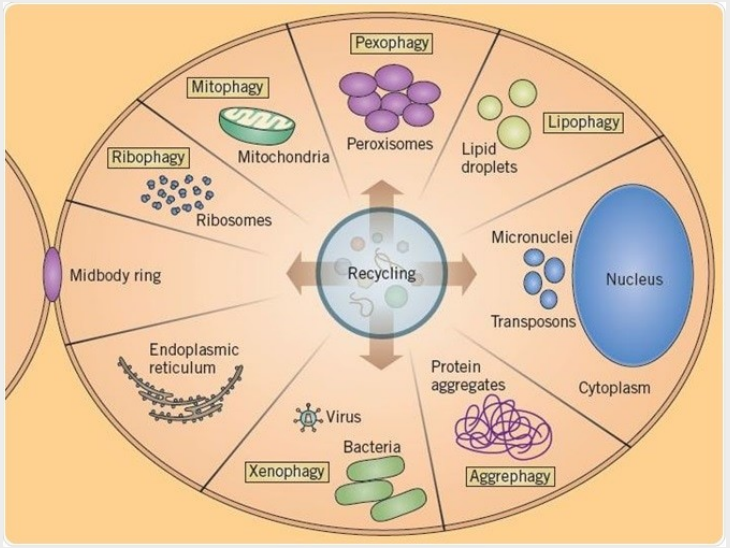
Ahmed A, Tait SWG. Targeting immunogenic cell death in cancer. Molecular Oncology. 2020;14(12):2994-3006.

Autophagy

The process of recycling cellular material, adaptation and maintenance of homeostasis of the internal environment of the cell.

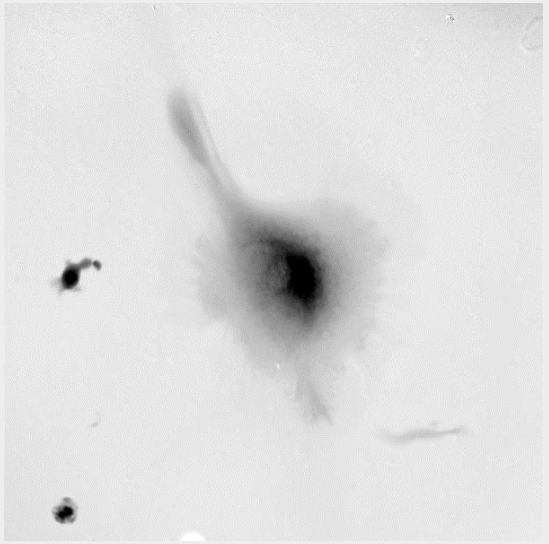
Under physiological conditions, it contributes to genome stability by regulating damaged proteins and organelles.
An important process in the differentiation of cells of the immune system and other tissues.
An important role in the adaptation of the newborn to oral food.
Disruption of this process is associated with many human pathologies: Neurodegenerative diseases (Alzheimer, Parkinson,…) - insufficient degradation of proteins by autophagy (eg. beta amyloid in the plaques of NS cells) is the cause of these diseases.

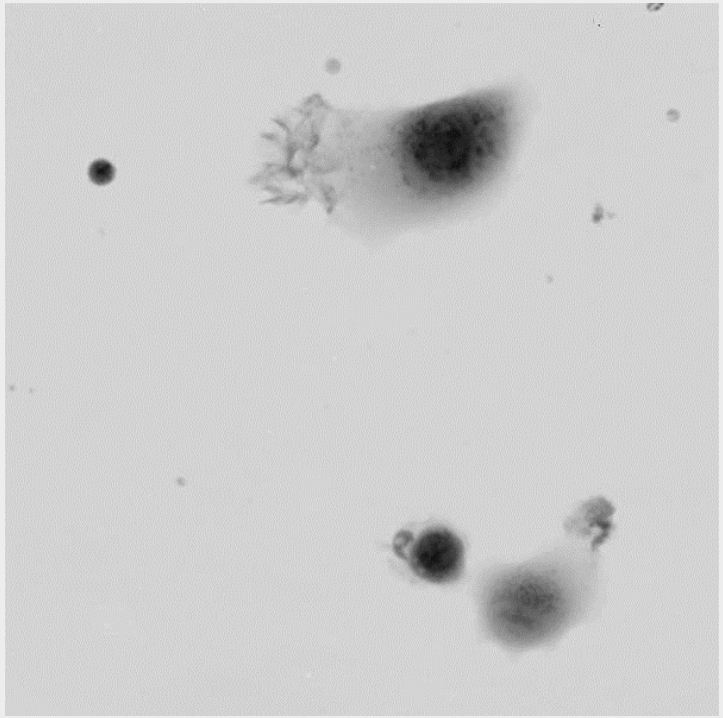


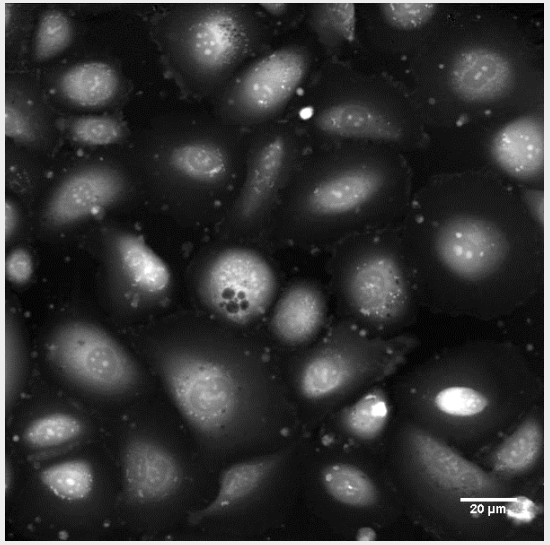


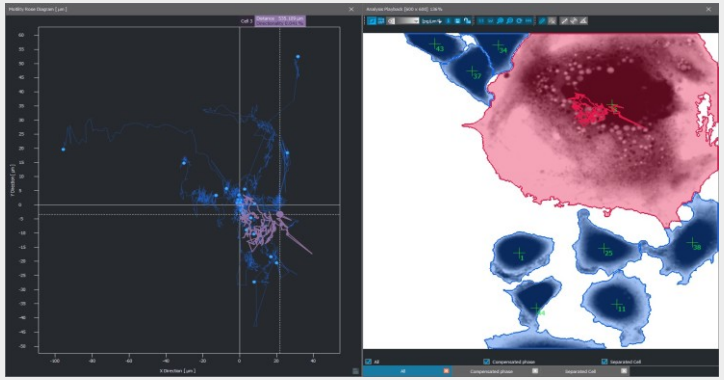
https://www.youtube.com/watch?v=Hqs1WzTwBEU&ab_channel=WallStreetJournal

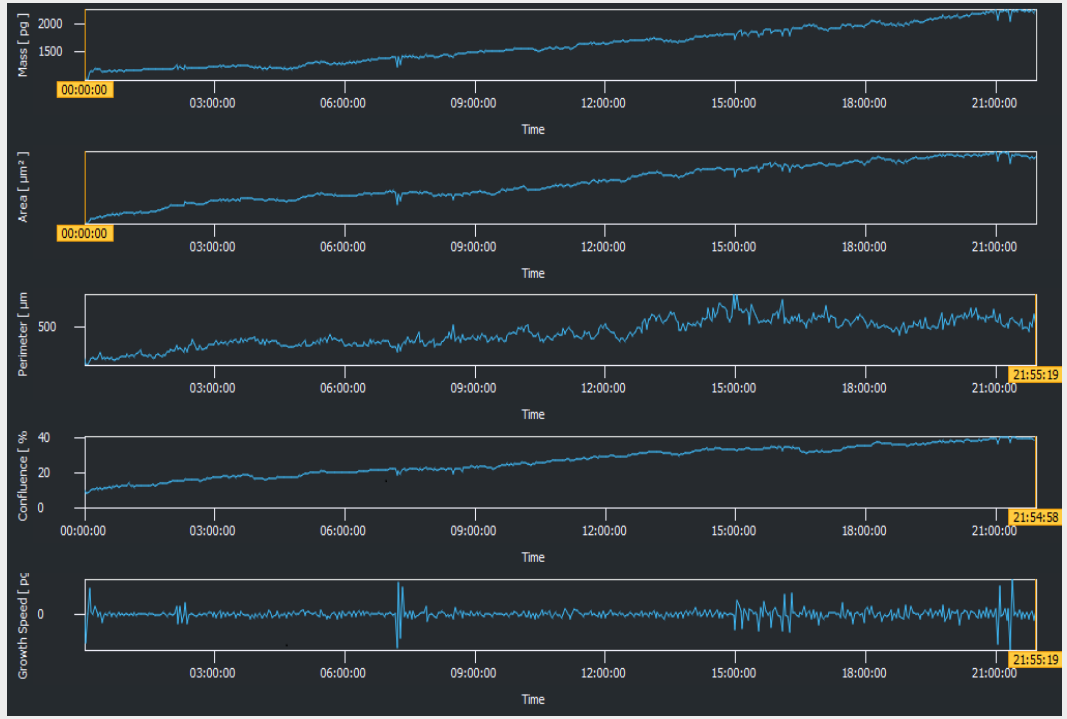
Holographic Microscopy and Quantitative Phase Imaging (QPI)





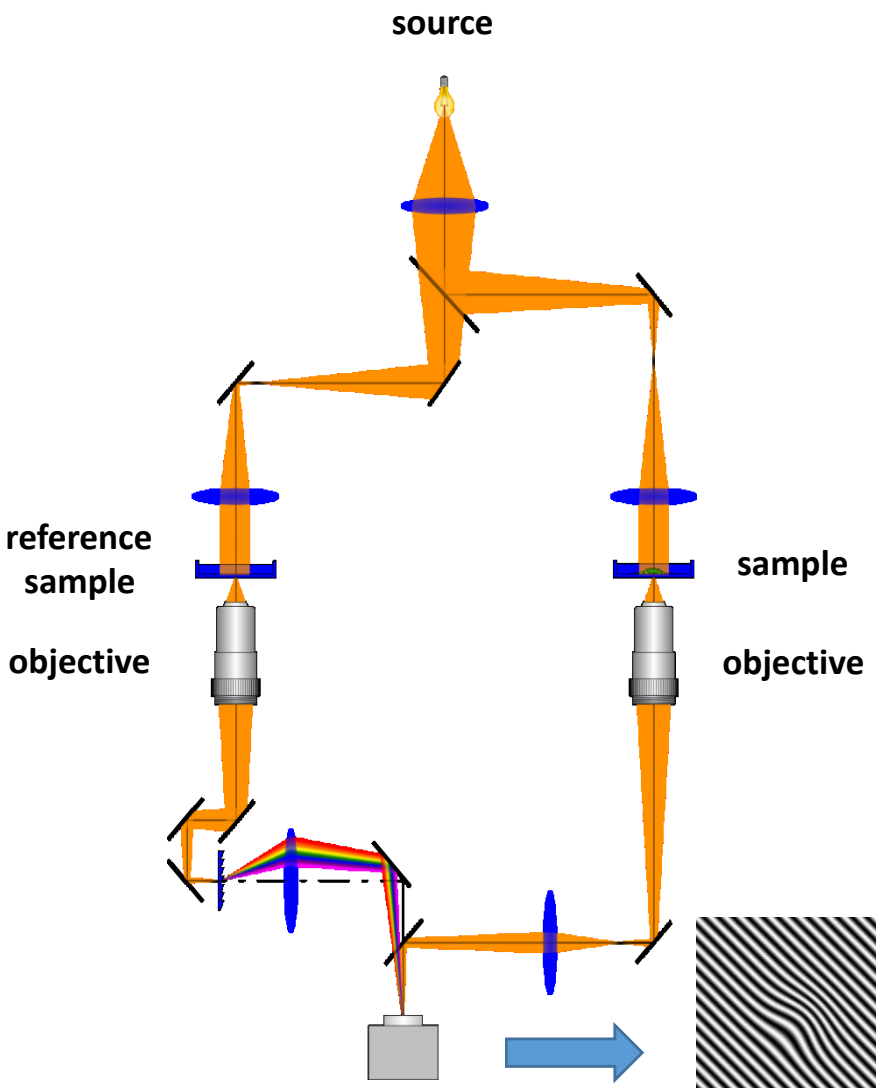




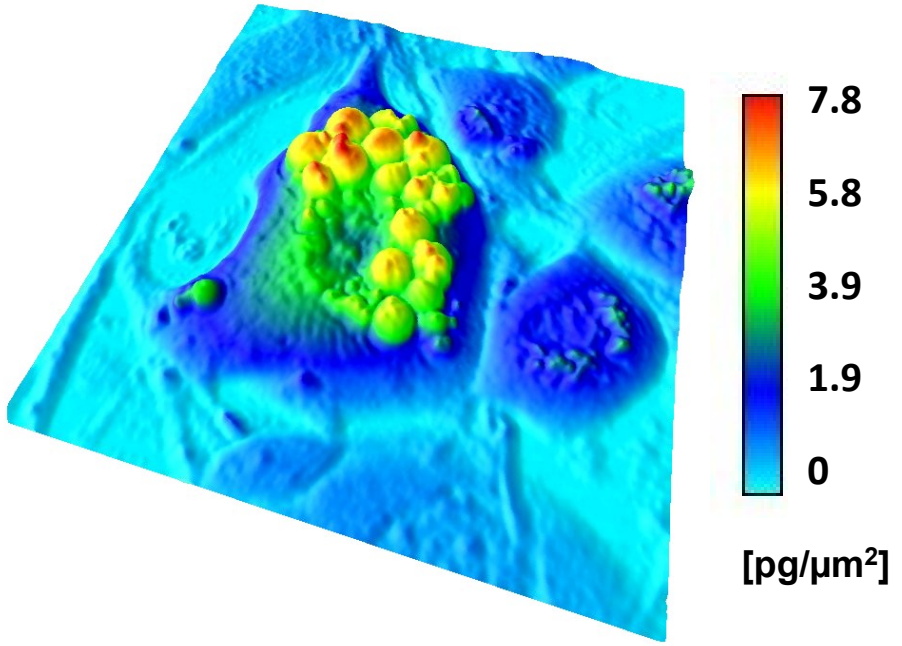


- **Long-term monitoring of the cell population**
- **Analysis of morphological and dynamic parameters in time**

Holographic Microscopy and Quantitative Phase Imaging (QPI)



- Beams from both arms are focused onto the CCD camera
- The beams interfere and form a hologram
- The hologram is recorded and further processed on PC to produce quantitativ phase image



Cell death detection using QPI

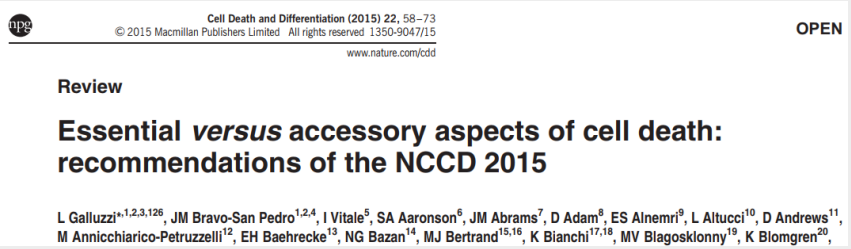
As a dead cell can be considered:

Cell whose membrane has lost its barrier function.

Cell which has disintegrated into separate bodies, often referred to as apoptotic bodies.

Cell which was engulfed by professional phagocytes or surrounding cells.

All these processes are associated with changes in cell mass!



Cell Death and Differentiation (2015) 22, 58–73

www.nature.com/cdd

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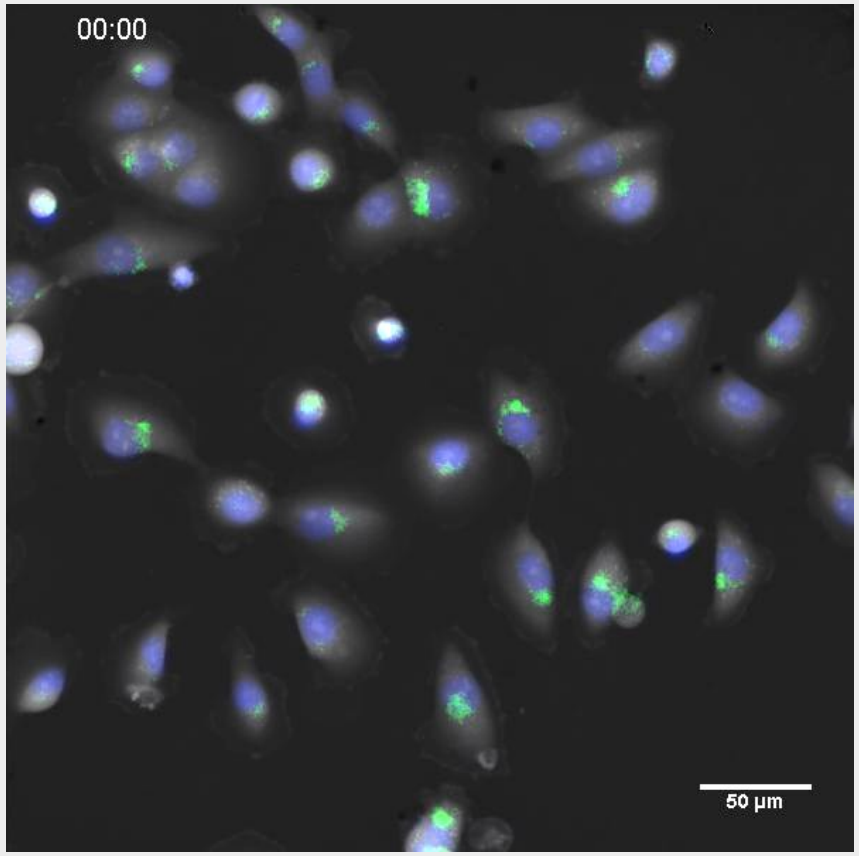
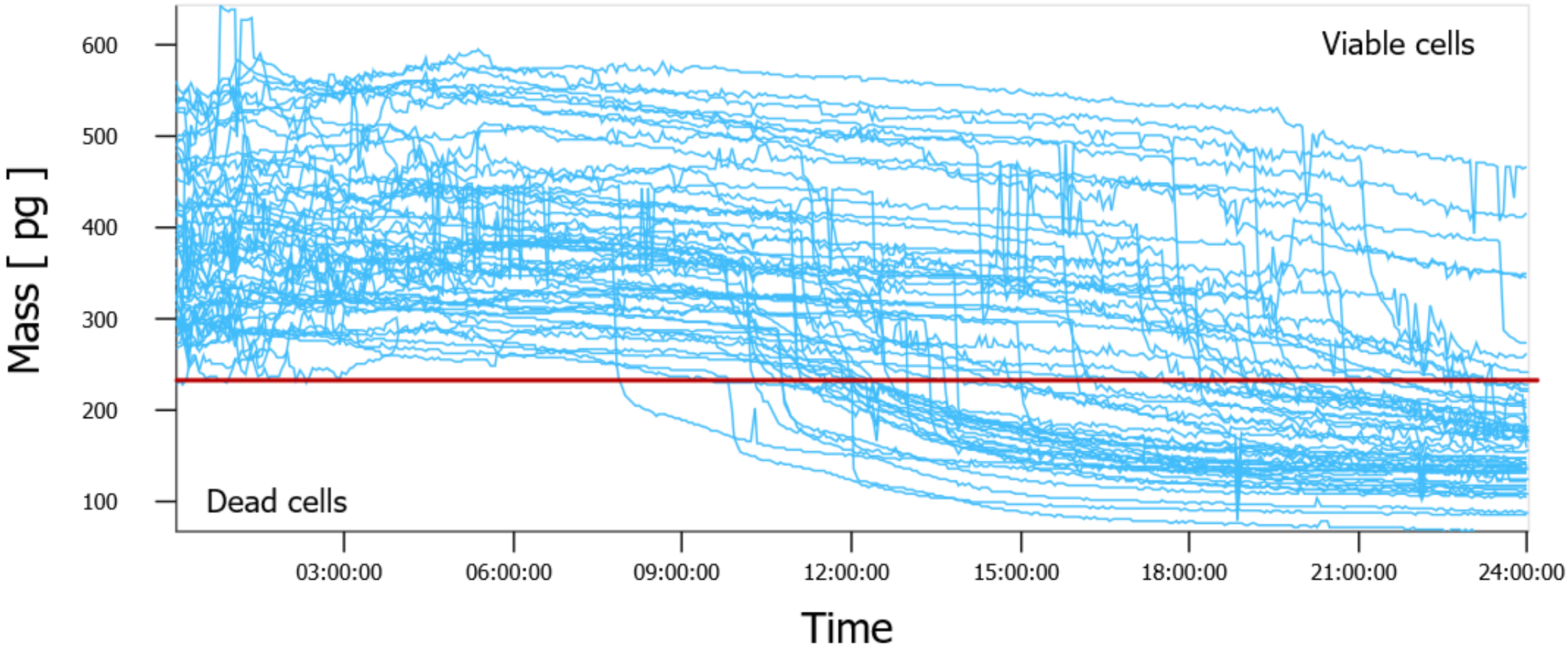
Review

Essential *versus* accessory aspects of cell death: recommendations of the NCCD 2015

L Galluzzi*[,1,2,3,126], JM Bravo-San Pedro[1,2,4], I Vitale[5], SA Aaronson[6], JM Abrams[7], D Adam[8], ES Alnemri[9], L Altucci[10], D Andrews[11], M Annicchiarico-Petruzzelli[12], EH Baehrecke[13], NG Bazan[14], MJ Bertrand[15,16], K Bianchi[17,18], MV Blagosklonny[19], K Blomgren[20],



Detekce buněčné smrti pomocí QPI



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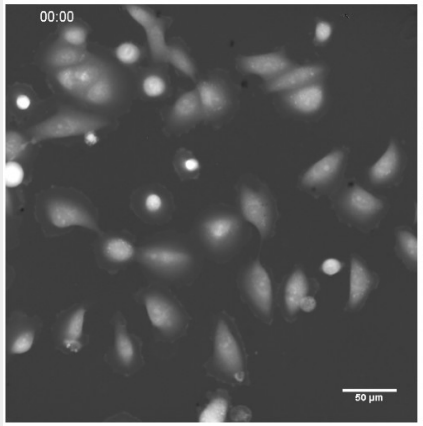
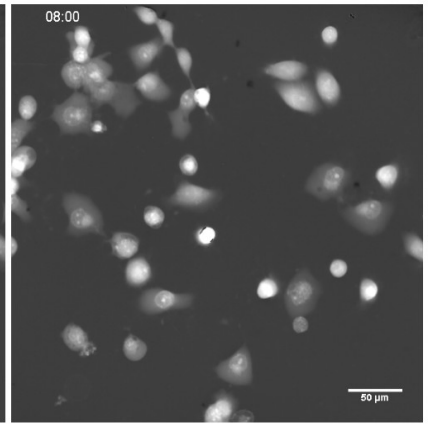
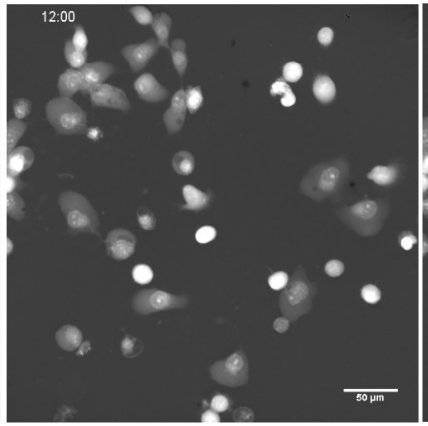
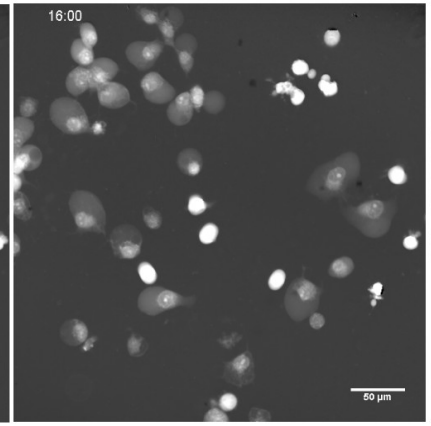
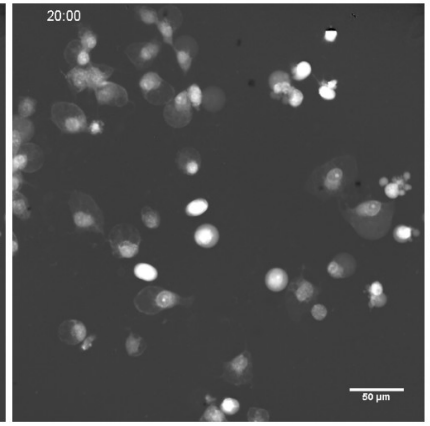
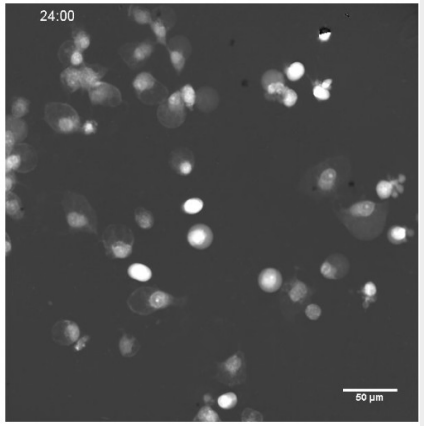


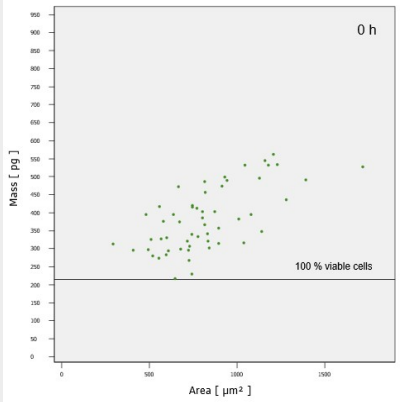


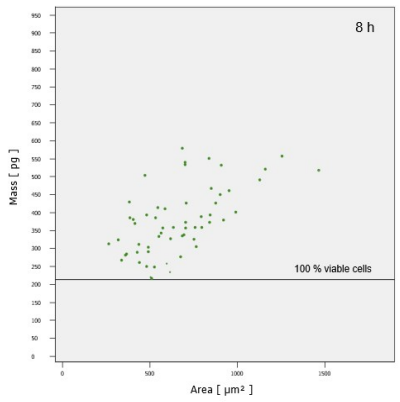


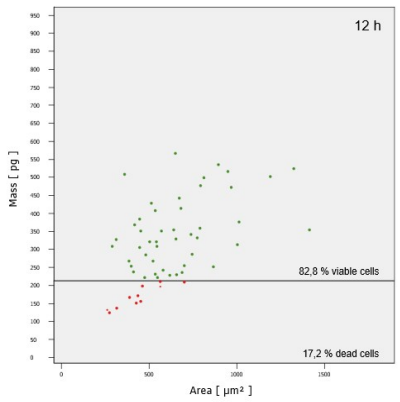


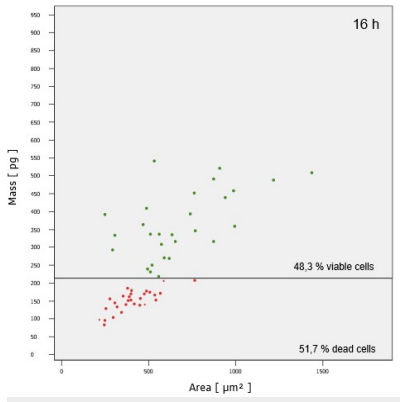


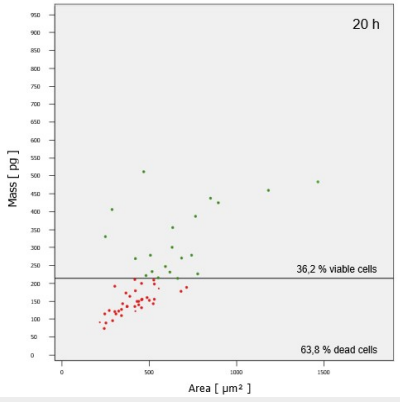


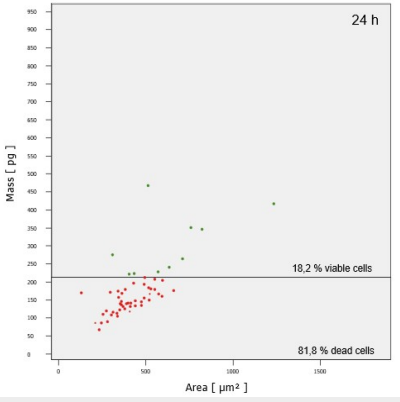






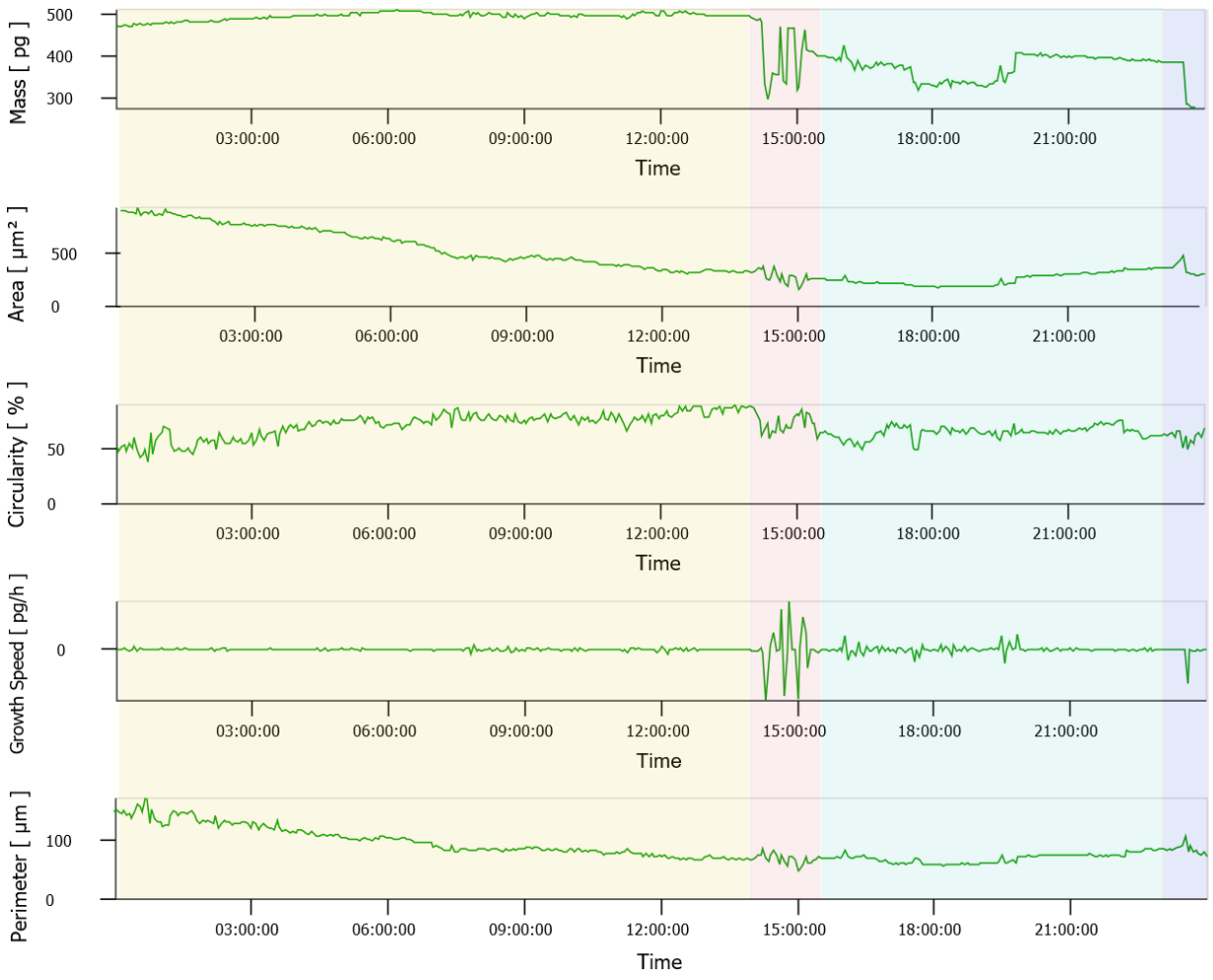




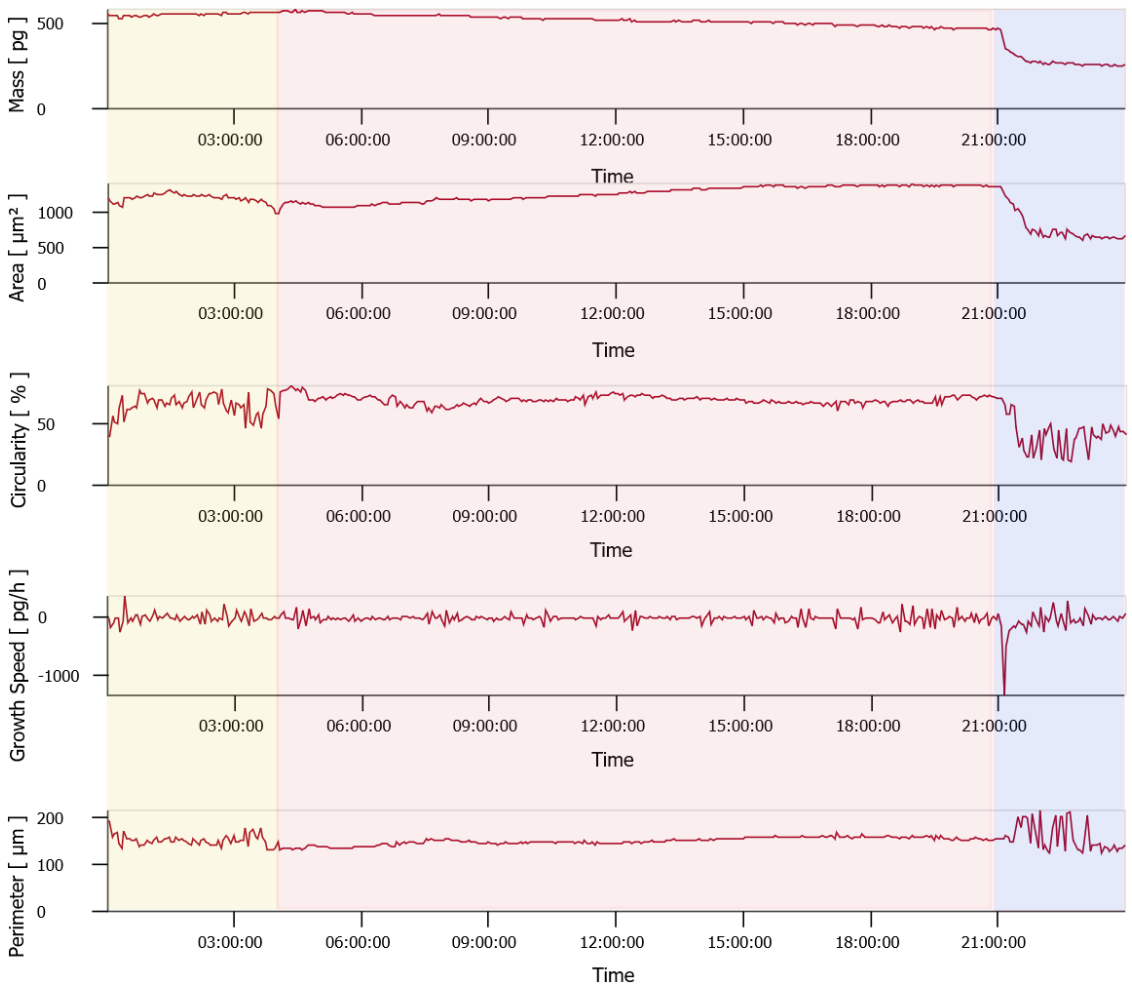


Rozlišení mezi apoptózou a nekrózou

Caspase-dependent (apoptosis)



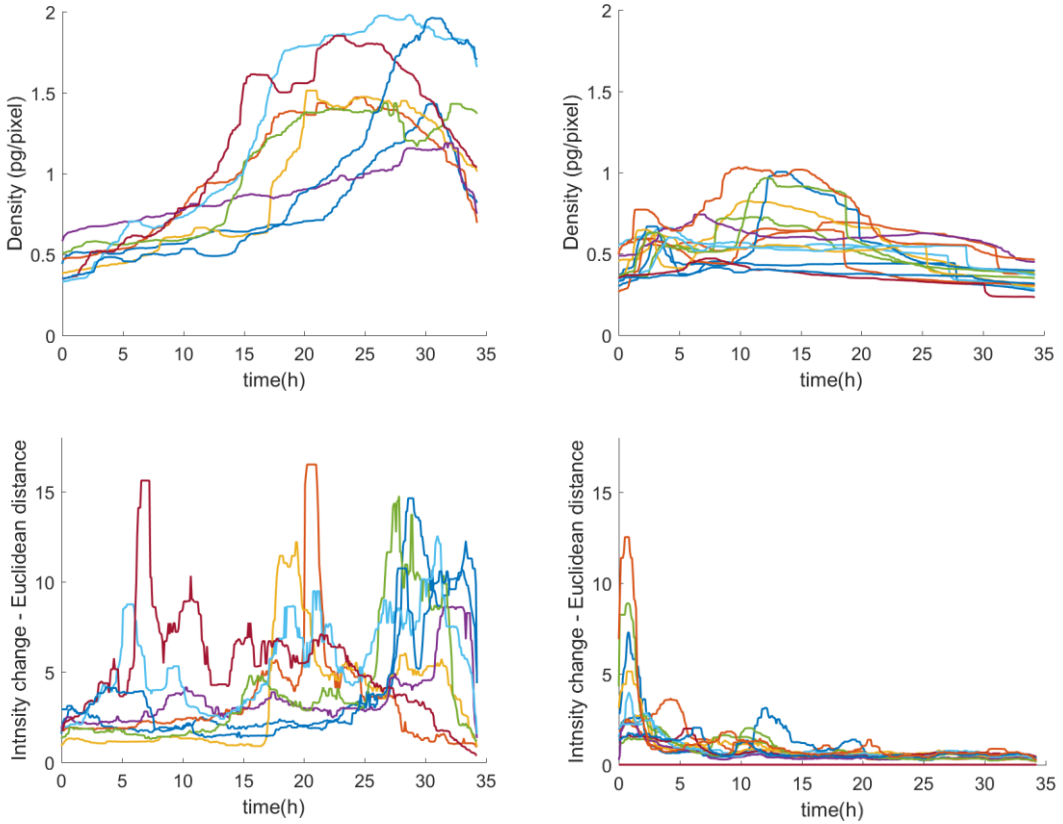
Caspase-independent (necrosis/necroptosis)

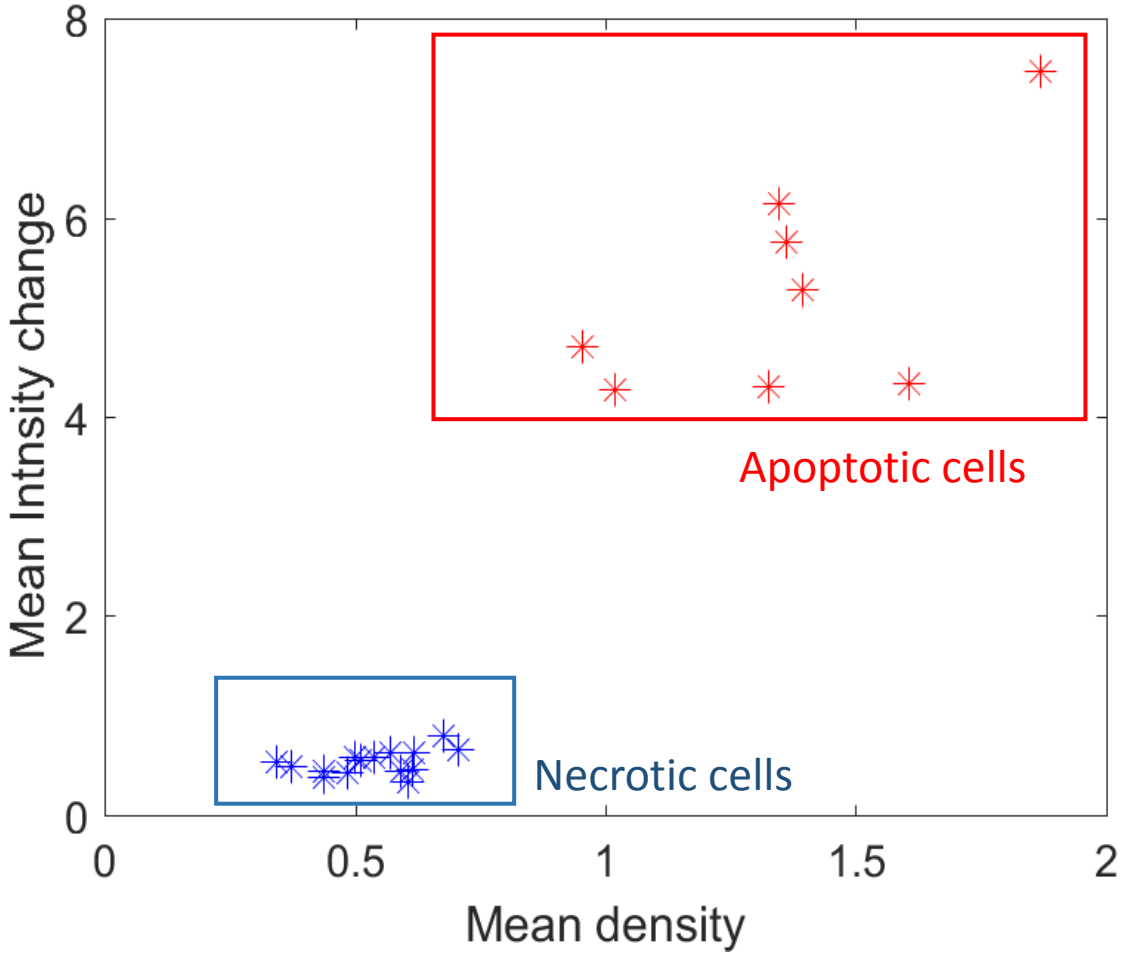


Rozlišení mezi apoptózou a nekrózou

Apoptotic cells

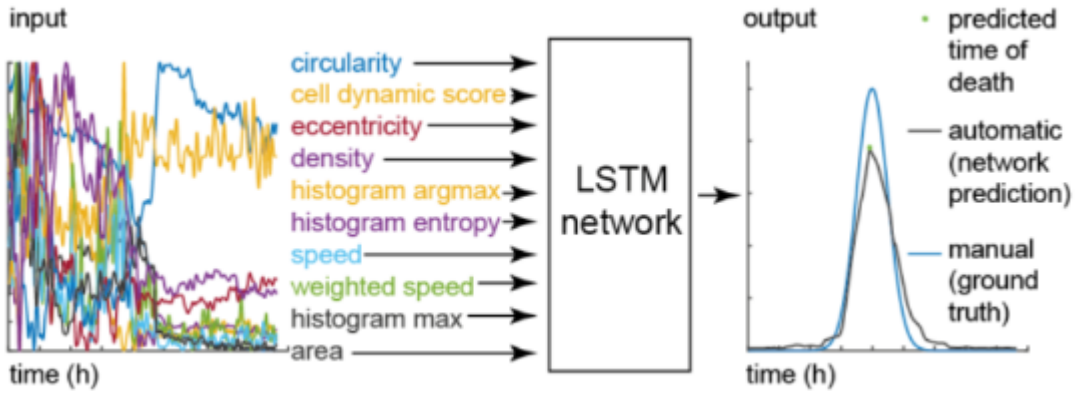
Necrotic cells

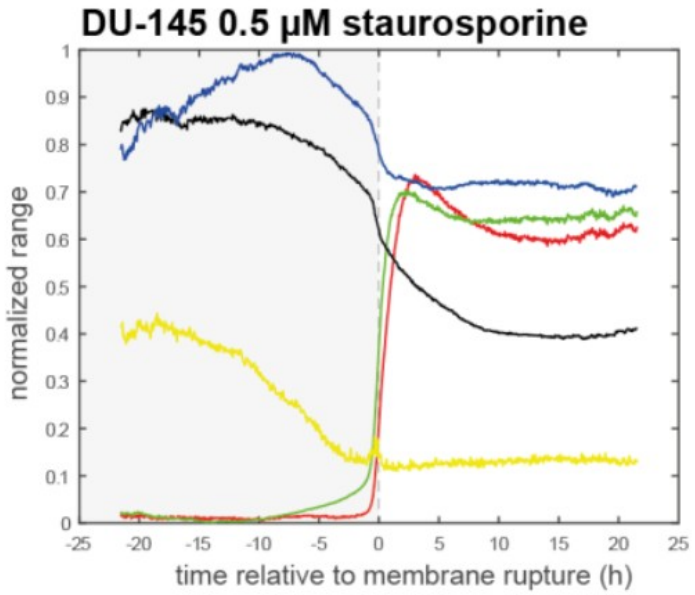


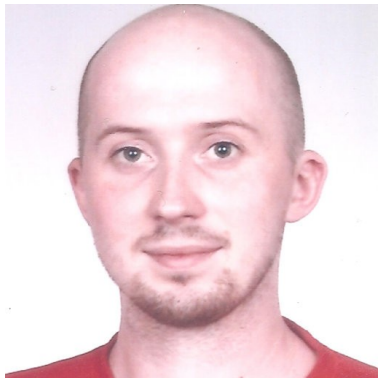


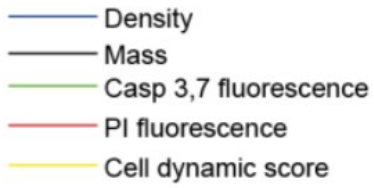
Based on morphological and dynamic parameters, we are able to automatically distinguish two distinct populations of cells. Without the use of dyes, only on the basis of a light microscopic method.

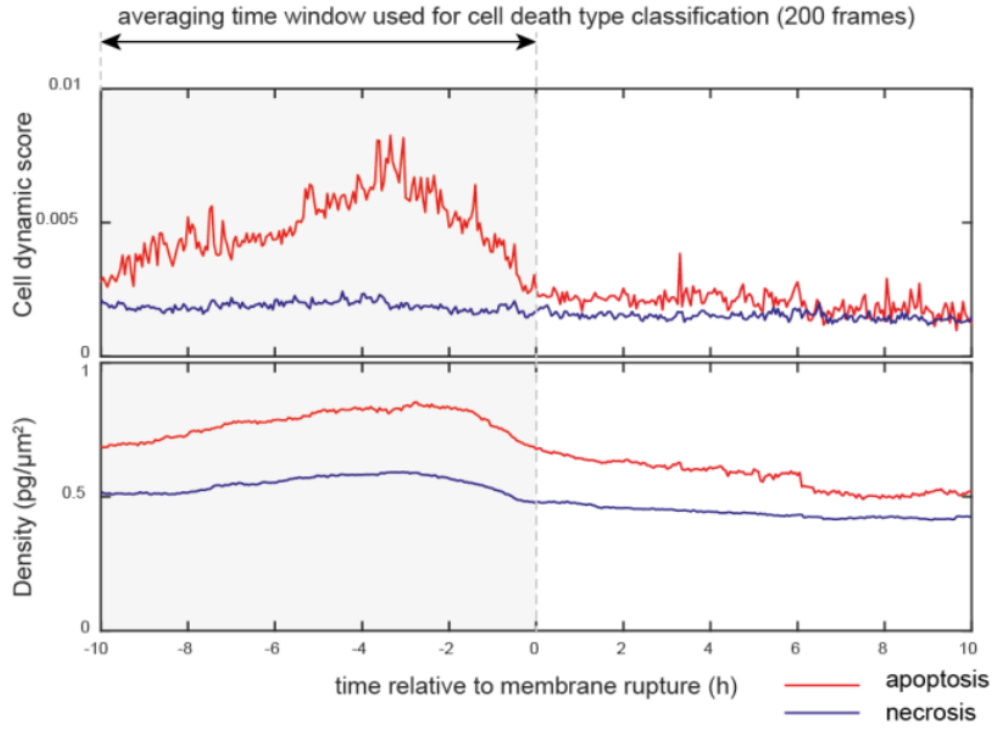
Rozlišení mezi apoptózou a nekrózou



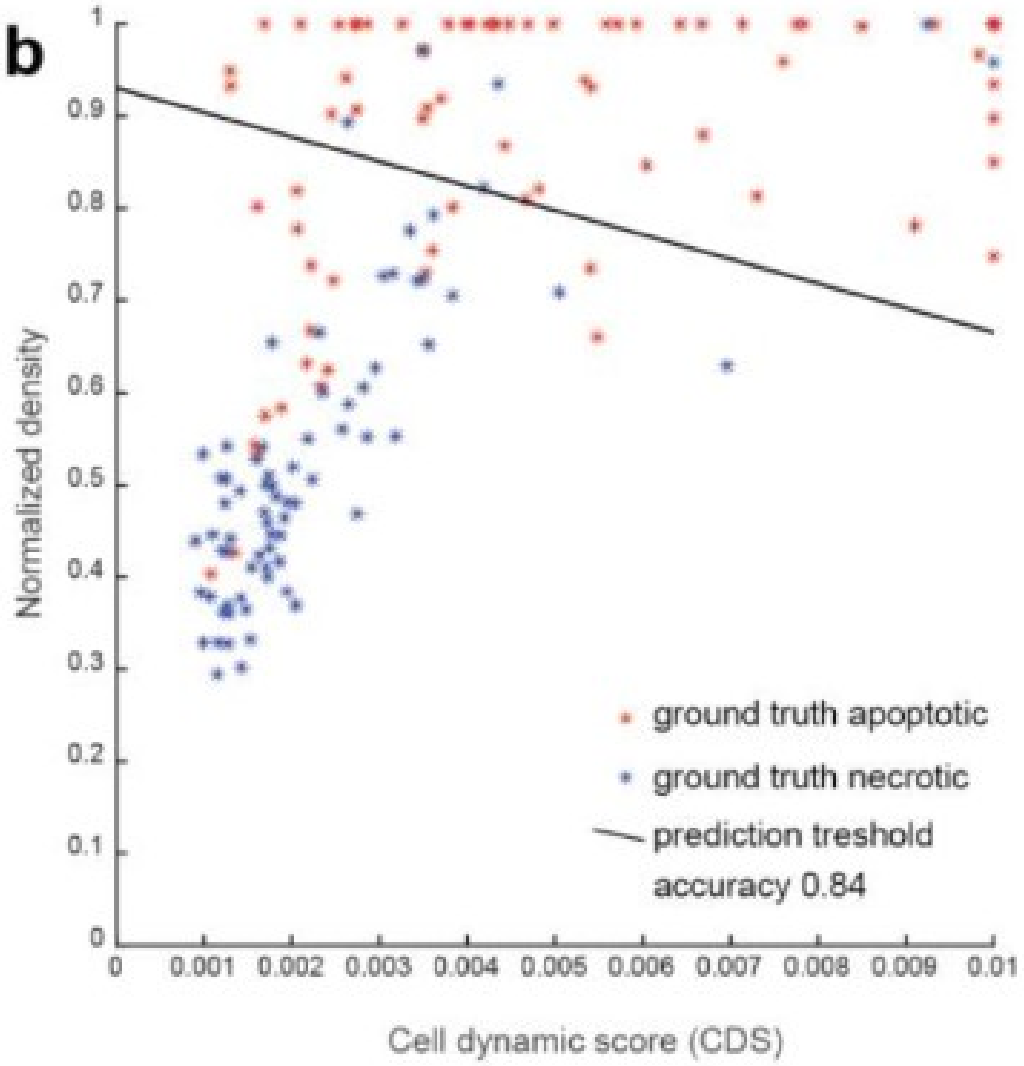


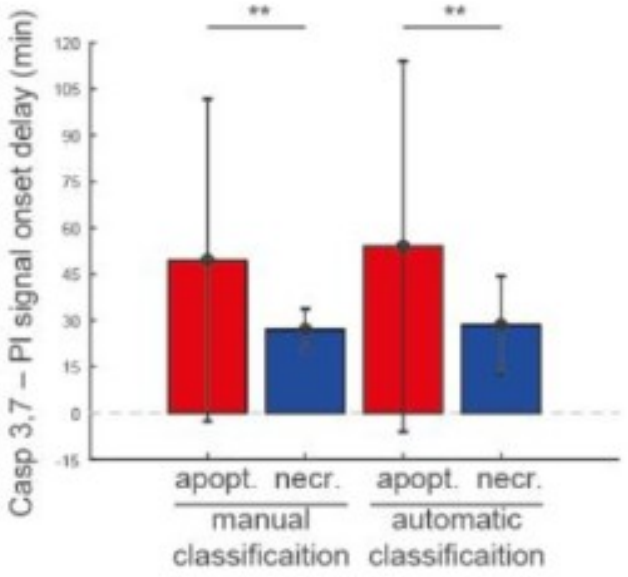


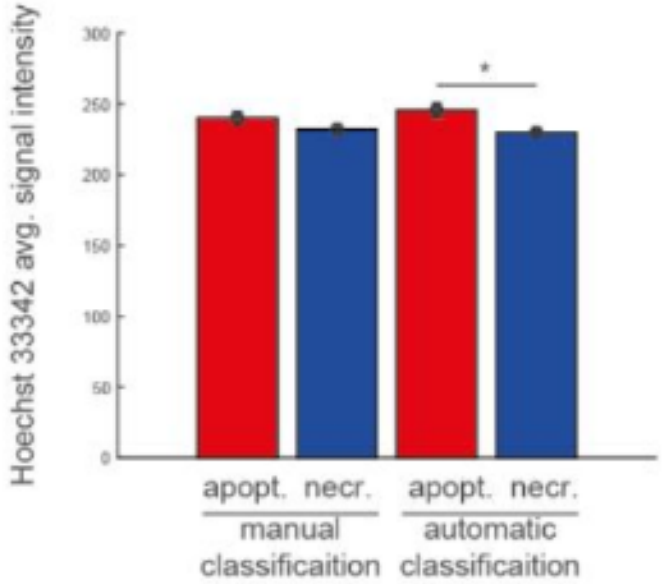


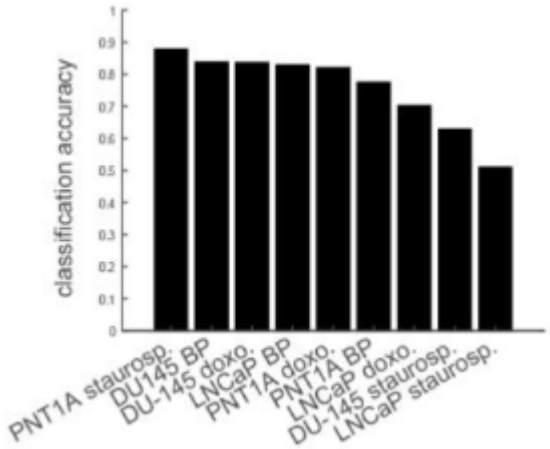


Rozlišení mezi apoptózou a nekrózou

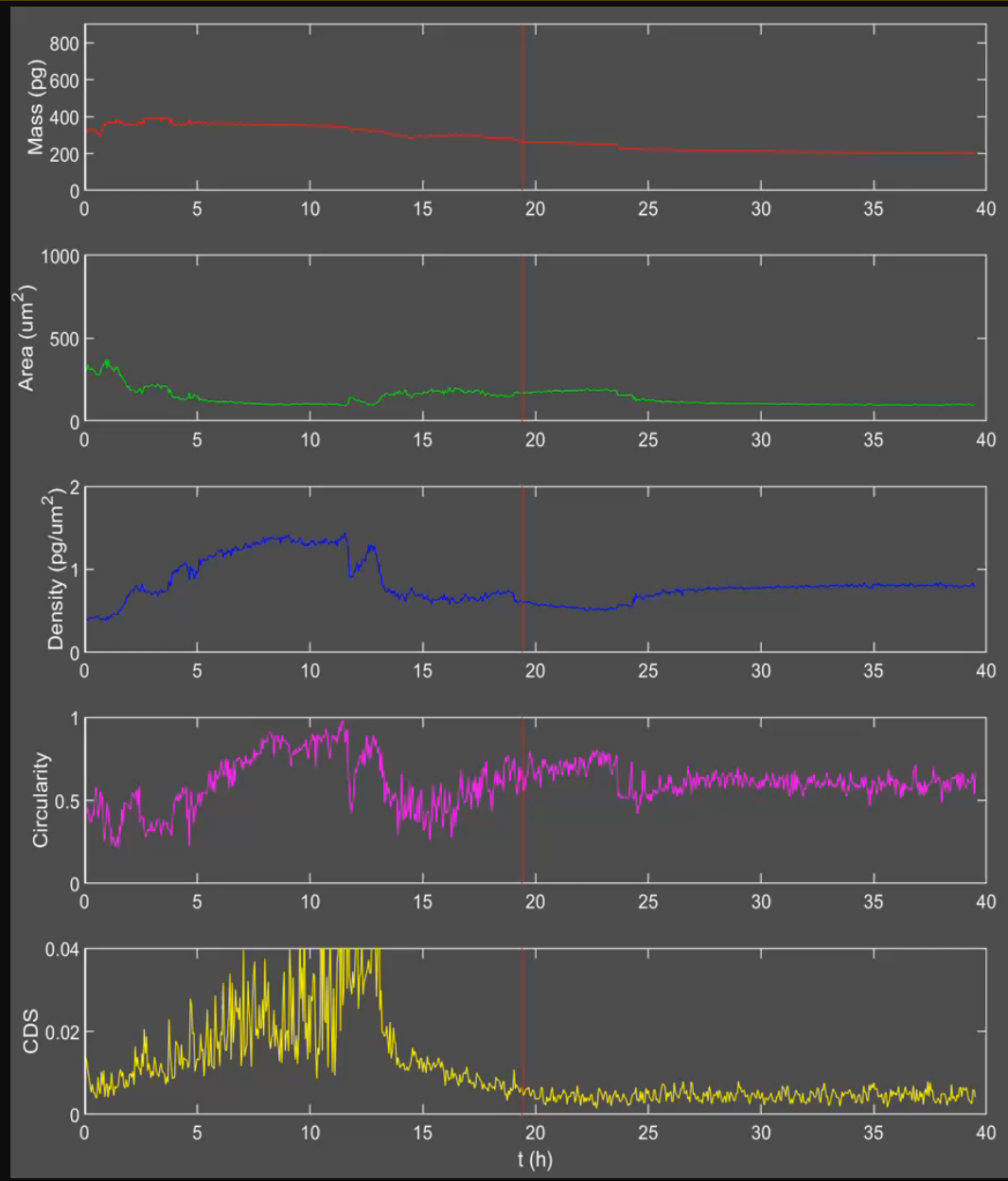


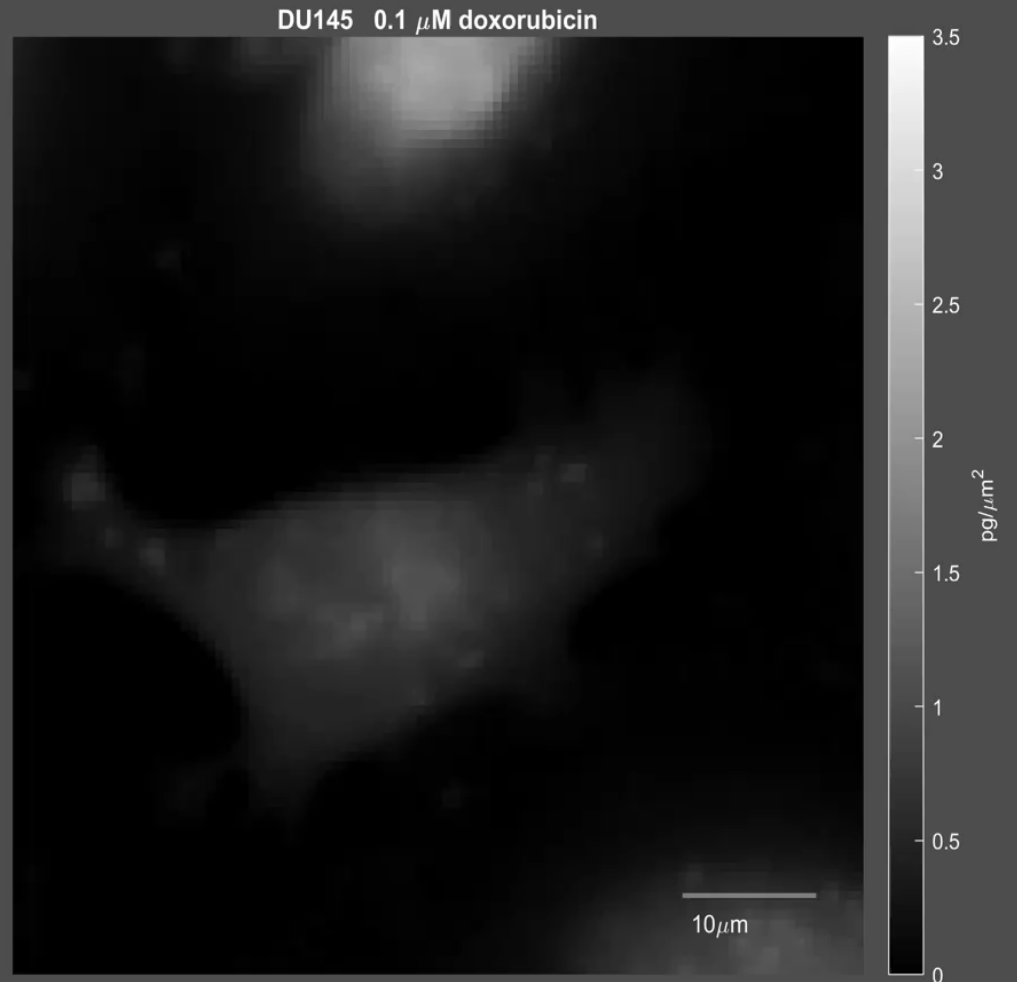






Rozlišení mezi apoptózou a nekrózou





Quantitative Phase Imaging, 10x magnification, 3 min/frame

apoptosis, morphologically cannonical (high density/high CDS)


Thanks for your attention.