Cell Death Classification with Machine Learning

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Cell Deaths

Cell death is a fundamental biological process essential for development, tissue homeostasis, and immune responses. Different types of programmed cell death serve distinct physiological functions and trigger varying immune reactions.

Non-Immunogenic Cell Death

- **Apoptosis**: "Silent" cell death with intact membrane, no inflammatory response

Immunogenic Cell Death

- **Pyroptosis**: Inflammatory cell death with membrane rupture

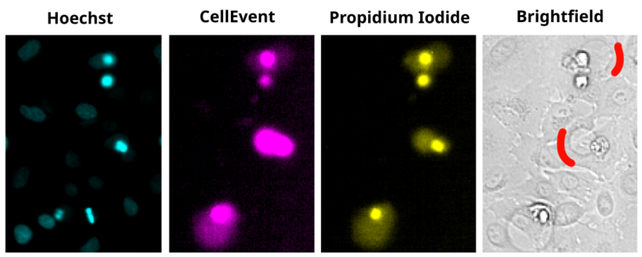

Fig. 1: Microscopic image containing pyroptotic cells

Why This Matters:

- **Apoptosis**: Cells die quietly, immune system does not learn about the threat, such as a viral infection
- **Pyroptosis**: Membrane breaks, releases cellular contents → innate immune system recognizes threat → adaptive immunity develops

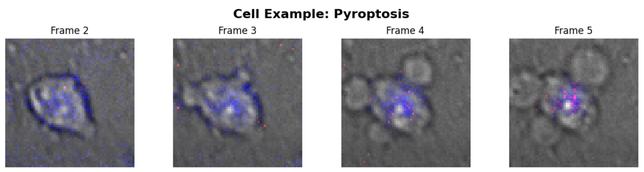

Fig. 2: Pyroptosis morphology

Current Approaches & Limitations

Methods of cell death detection

- **Fluorescent markers** that translocate to dying cells (e.g., propidium iodide) or are triggered by a specific death pathway
- **Cell morphology** analysis

Key Problems:

- **Marker constraints** – using fluorescent markers for death detection limits the use of them for other signaling.
- **Pathway specificity** – most tools only detect apoptosis or general cell death
- **Resolution limitations** – whole-image classification rather than single-cell detection
- Very few methods specifically detect **pyroptosis**

Dataset Creation

Data Sources & Experiment Design

- **Live-cell time-lapse microscopy** of viral infection experiments
- **6 independent experiments** on epithelial cells,
  - infected with influenza,
  - stimulated with viral RNA analog, poly(I:C),
  - co-incubated with natural killer cells

  all leading to cell death.
- **Reference standard**: Propidium iodide staining for cell death confirmation

Available Channels

- **Brightfield (BF)**: Cell morphology in white light
- **CellEvent (CE)**: Caspase-3 activation indicator
- **Propidium Iodide (PI)**: Dead cell indicator
- **Cell Nuclei**
- **Differential Phase Contrast (DPC)**

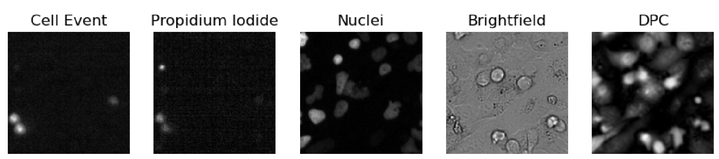

Fig. 3: Example channels visualization

Created Dataset Types

Dataset Type	Description	Size (train / test)
2D Classification	Single timepoint crops containing cell of interest	7317 / 1830
3D Classification	9 consecutive frames (*t-4* to *t+4*)	1601 / 403
Object Detection	Full field images with annotations	2476 / 619

Annotation Process

1. **Pyroptosis identification**: Human annotation based on PI signal and morphological criteria
2. **Ground truth**: PI channel provides cell death information
3. **Temporal tracking**: Cells tracked across time

single snapshot clasification with 2D CNN

Training Strategy: Classification of single-timepoint images (224 by 224 pixels) containing the cell of interest and its surroundings.
The network (ResNet-18, EfficientNet-B0) is trained to classify the input into

- **Binary**: Pyroptotic vs. Non-pyroptotic
- **3-class**: Pyroptotic vs. Apoptotic vs. Healthy/Other

The network is trained on all channels or one chosen channel.

Snapshot sequence classification with 2D CNN+LSTM

- **ResNet + LSTM**: 2D feature extraction followed by temporal modeling by LSTM layer
- 9-frame sequences centered on target timepoint
- 64×64 pixel frames

Classification Schemes

- **Binary**: Pyroptotic vs. Non-pyroptotic
- **3-class**: Pyroptotic vs. Apoptotic vs. Healthy/Other

Results

Three class classification

Approach	Backbone	Channels	Accuracy (%) (mean)
2D CNN	ResNet-18	All	95.9
2D CNN	ResNet-18	Brightfield	82.30
2D CNN	ResNet-18	PI	89.34
2D CNN	ResNet-18	CE	85.74
CNN+LSTM	ResNet-18	All	94.29
CNN+LSTM	ResNet-18	Brightfield	76.59
CNN+LSTM	ResNet-18	PI	91.07
CNN+LSTM	ResNet-18	CE	82.13

Binary classification (Pyroptotic vs. others)

Approach	Backbone	Channels	Accuracy (%)
2D CNN	ResNet-18	All	96.39
2D CNN	ResNet-18	Brightfield	89.96
2D CNN	ResNet-18	PI	84.04
2D CNN	ResNet-18	CE	89.67
CNN+LSTM	ResNet-18	All	88.09
CNN+LSTM	ResNet-18	Brightfield	84.29
CNN+LSTM	ResNet-18	PI	89.0
CNN+LSTM	ResNet-18	CE	88.34

Where to find the datasets?

Datasets are available on HuggingFace:

2D Classification

kaczyniec/pyroptosis-classification-2d

3D Classification

kaczyniec/pyroptosis-classification-3d