# UNDERSTANDING CELLULAR DECISIONS: OLD AND NOVEL MICROSCOPY TOOLS

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## 1. BACKGROUND

At any given time in adulthood, our bodies are made of 3 trillion cells. These cells have to continually maintain tissue function — our skin protecting us from the environment, the lungs giving us oxygen, the heart pumping blood through our veins. Every day, 1 billion of these cells die and have to be replenished. By the end of our lifespan, we shed 30 trillion cells. This means that, at any given time, cells have to make choices such as dying, dividing, moving and becoming a different cell type to maintain our bodies. A vast amount of cell decisions is taken at any given moment. Rarely, some cells take a wrong decision that may trigger a cascade of events resulting in cancer, the uncontrolled growth of cells within an organ of our body. Cancer cells will then keep taking decisions outside the rulebook that make our bodies the amazing machines they are.

## 2. OLD AND NEW MICROSCOPY TOOLS TO UNDERSTAND CELL CHOICES

In this talk, we will exemplify how a range of microscopy techniques can and has to be used to better understand cellular decisions. We'll focus on the DNA damage response, a complex sequence of events required to repair DNA damage. For this purpose, we developed novel fast multi-colour FLIM with multi-dimensional phasor fingerprinting to permit mapping the biochemical networks underlying cell fate choices such as apoptosis and necrosis. This technique is permitting us to image three FRET sensors at the same time. Furthermore, we have applied optogenetics for the light-inducible control of KRAS-dependent signalling and FRET-based detection of ERK signalling [1], to understand how cells establish a balance between cell death and survival. We used FUCCI imaging [2], for the understanding of how a cell that escape cell death with damaged DNA gives rise to a lineage of cycling cells that may help cancer to escape therapies. We also used super-resolution microscopy [3] and fluorescence correlation spectroscopy [4] to reveal the choreography of DNA damage repair proteins (*e.g.*, RPA, RAD51 and BRCA2) that occurs at the site of DNA damage.

## 2. CONCLUSIONS

In face of similar DNA damage, some cell die by apoptosis, some by necrosis, other will trigger senescence, or repair DNA damage successfully, or progress in the cell cycle with DNA damage. This considerable cell-to-cell variability is matched by a significant heterogeneity in those biochemical networks that encode for cellular decisions. It is only light microscopy in its various incarnations that, by preserving the integrity and identity of single cells, and offering high spatial [3] and biochemical resolution [5], spanning from established techniques to the latest innovations, will permit scientist to model the complexity of cellular decisions, with invaluable insights on how cells work in normalcy and disease.

**[1]** Aoki *et al.*, *Mol Cell* **52**(4), 529-40 (2013); **[2]** Sandler *et al.*, *Nature* **519**, 468-71 (2015)
**[3]** Haas *et al.*, *Nucleic Acids Res* **e-print** (2018) **[4]** Jeyasekharan *et al.*, *PNAS* **107**(50), 21937-42 (2010); **[5]** Esposito *et al.*, *PLoS One* **8**(10):e77392 (2013)