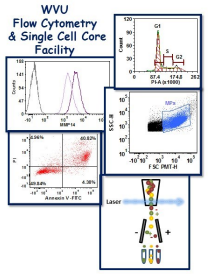
WVU FLOW CYTOMETRY & SINGLE CELL CORE FACILITY



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Measuring Cellular Senescence Using Flow Cytometry

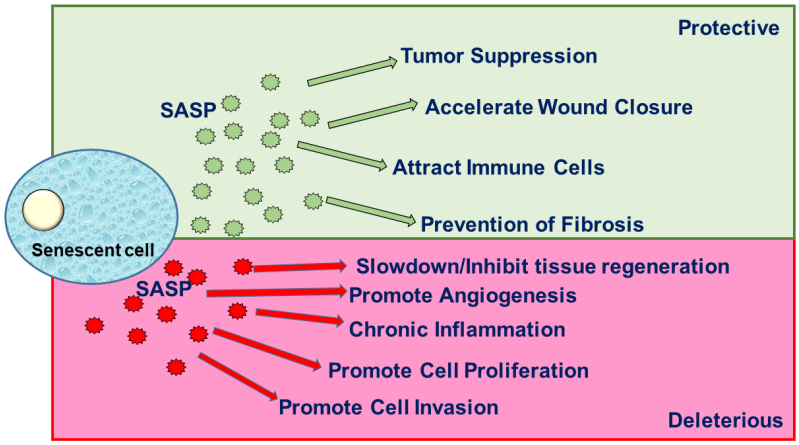

Figure 1. Processes affected by senescence cells

What is senescence?

When a cell is in a stable state of cell cycle arrest but is still metabolically active, and resistant to growth promoting stimuli, it is said to be in senescence. A senescent cell is characterized as having morphological and metabolic changes, chromatin reorganization, altered gene expression, and in many instances a pro-inflammatory phenotype. These cells are distinct from quiescent cells which can reenter the cell cycle. Senescent cells are also different from terminally differentiated cells as they don't respond to stimuli.

It has been hypothesized that senescence evolved as a mechanism to prevent damaged cells from becoming malignant. However, research has shown that this is not the whole story. Over the thirty years or so, senescent cells have been demonstrated to have a role in cancer, tissue degeneration, inflammatory diseases and aging. The currently thinking is that, senescence cells have the potential to have both protective and deleterious effects (Figure 1).

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Hours of operation:
9:30 am to 5:00 pm, M-F

After hours access is available for experienced users with prior approval from
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What is SASP?

This pro-inflammatory phenotype associated with senescence is known as a senescence associated secretory phenotype or SASP for short. SASP of senescent cells is comprised of a mixture of secreted cytokines, chemokines, growth factors and proteases. The exact composition depends on the cell type, tissue, and stimuli. SASP can effect many processes (Figure 1). It can have a tumor suppression function, recruiting immune cells that will eliminate the senescent cell. It can promote tumors by secreting pro-angiogenesis factors, remodel the extracellular matrix or promote epithelial-mesenchymal transition. SASP can also induce inflammation causing tissue damage and age related tissue degradation as well as promoting cancer.

How do cells become senescent?

Senescence can be induced in several ways including telomere shortening, DNA damage, and oncogenesis. In vitro, after approximately 50 cell divisions, nonmalignant cells will stop dividing due to the shortening of their telomeres. These shortened telomeres induce a DNA damage response that triggers senescence. Both the amount of DNA damage as well as the physiological context will determine if a cell will enter senescence. Ionizing radiation, chemotherapeutics, genotoxic stress and oxidative stress can all induce DNA damage that results in a cell becoming senescent. Oncogene induced senescence has been shown to occur as the result of hyperactivation of oncogenes such as H-RAS or inactivation of tumor suppressor genes such as PTEM.

How do you identify a senescent cell?

Since many of the markers that identify a cell in senescence are also found in non-senescence cells it is important to measure several different markers when determining if a cell has entered senescence or not. One of the hallmarks of senescent cells is stable cell cycle arrest even in the presence of stimuli. Cell cycle arrest is controlled by p53/$p21^{CIP1}$ and $p16^{INK4A/pRb}$. Often researchers will assay $p16^{INK4A/pRb}$ levels as a measure of senescence cell cycle arrest keeping in mind that high levels of $p16^{INK4A/pRb}$ is also found in pRB negative tumors and cell lines.

There are many morphological as well as metabolic changes in a senescent cell when compared to a cell that is not in senescent. Under a microscopy, senescent cells tend to be relatively flat and enlarged with many vacuoles compared to non-senescent cells. In addition, in many instances they will be multi-nucleated. In addition, senescent cells show a loss of lamin B1 in the nuclear envelope resulting in a loss of nuclear membrane integrity. Senescent cells appear to accumulate a lot of dysfunctional mitochondria along with having increased levels of reactive oxygen species (ROS).

In senescent cells, there is an increase in lysosomal content as well as alterations in lysosomal activity. These changes to the lysosomal compartment of the senescent cell can be observed by measuring β-galactosidase activity at pH 6.0. Senescent cells have increased levels compared to non-senescent cells.

Chromatin reorganization with altered gene expression is another way to identify senescent cells. Some senescent cell have been shown to have senescence-associated heterochromatin foci (SAHF). SAHF has been demonstrated to have a role in silencing genes that promote proliferation such as E2F targeted genes. The SAHFs can be detected microscopically using DAPI (brightly stained),

macroH2A, heterochromatin protein 1 (HP1) and lysine 9 di– or tri-methylated histone H3 (H3K9Me2/3).

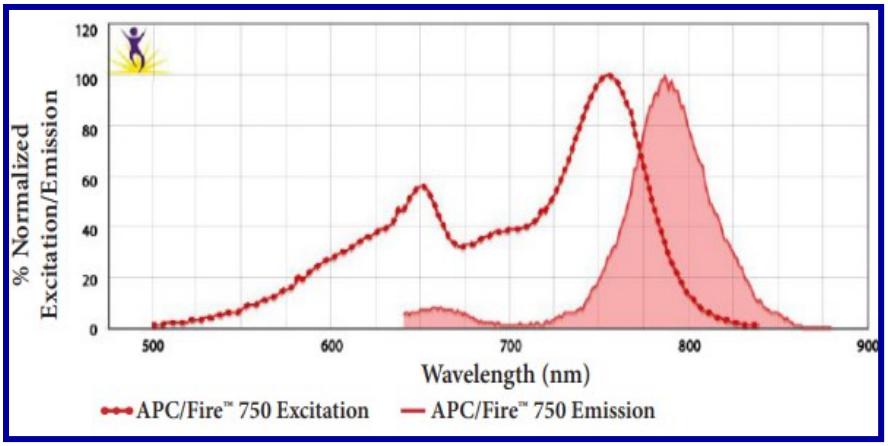
Double stranded DNA breaks are another common feature of senescent cells. Measurement of phosphorylated H2A.X (histone protein) is another common assay for determining if a cell is in senescence. Increased/high levels of phosphorylated H2A.X is a good indication that the cell has a number of double stranded DNA breaks.

Kit Name	Manufacturer	Catalog Number	Assay
Senescence Assay Kit	**Abcam**	**ab228562**	**Detects β-galatosidase activity**
CellEvent Senescence Green Flow Cytometry Assay Kit	**Thermo Fisher**	**C10840**	**Detects β-galatosidase activity**
Quantitative Cellular Senescence Assay Kit	**Cell Biolabs**	**CBA-232**	**Detects β-galatosidase activity**
Cellular Senescence Live Cell Analysis Assay	**Enzo**	**ENZ-KIT130-0010**	**Detects β-galatosidase activity**
H2A.X Phosphorylation Assay Kit (Flow Cytometry)	**Millipore Sigma**	**17-344**	**Measures the level of phosphorylated H2A.X**

Using Flow Cytometry to Identify Senescent Cells

Shown in the table above are some of the kits that use flow cytometry to measure markers associated with senescent cells. The majority of the available kits look at the lysosomal compartment. These kits measure β-galactosidase activity using a green fluorescent probe readout, with increase fluorescence indicating increase β-galactosidase activity and the possibility that the cells are in senescence. There is also a kit that looks at double stranded DNA damage by measuring the level of phosphorylated H2A.X in the cells. As with the β-galactosidase activity, an increase in phosphorylated H2A.X is indicated of a cell in senescence. Regardless of what assay you use to determine if you r cells are senescent, it is important to looks at several biological characteristics before identifying your cells as being in senescence.

Meet the Fluorochrome: APC-Fire750 from Biolegend



Excitation

633 nm (Red laser)

Emission max

780 nm (same detector as the one used for APC-Cy7)

Type of Fluorochrome: Tandem Dye

Characteristics: As bright as APC-Cy7; brighter than APC-H7
More stable than APC-Cy7
Low spillover into other detectors

Flow Cytometers in the Facility

FACSAria III Cell Sorter

Operator: Facility Staff

Lasers:

- **488 nm solid state**
- **561 nm solid state**
- **633 nm solid state**
- **407 nm solid state**

Detection Parameters:

- **Forward Scatter**
- **Side Scatter**
- **Simultaneous detection of 13 fluorochromes**

Applications:

- **Cell Sorting (Aseptic)**
- **Single Cell Sorting**
- **Cell Phenotyping**
- **Cell Viability**
- **Cell Cycle Analysis**

Flow Cytometers in the Facility (continued)

LSR Fortessa

Operator: User or Facility Staff

Lasers:

- 405 nm solid state
- 488 nm solid state
- 561 nm solid state
- 628 nm solid state

Detection Parameters:

- Forward Scatter
- Side Scatter
- Simultaneous detection of 17 fluorochromes

Applications:

- Cell phenotyping
- Cell Viability
- Cell Cycle analysis
- Apoptosis Assays

Guava easyCyte HT

Operator: User or Facility Staff

Lasers:

- 488 nm solid state

Detection Parameters:

- Forward Scatter
- Side Scatter
- Simultaneous detection of 3 fluorochromes

Applications:

- Cell Counts
- Apoptosis Assay
- Cell Cycle Analysis

Other Instrumentation Available in the Facility

AutoMACS Pro Magnetic Bead separator

Operator: User

Application:

- Single extracellular marker cell sorting
- Depletion/negative cell sorting

gentleMACS Octo Dissociator with Heaters

Operator: User

Application:

- Dissociation of tissues into single cell suspension for culture or flow cytometry assays
- Homogenizes tissues for downstream molecular biology applications

C1 Single Cell Auto Prep System

Operator: User or Staff

Application:

Uses microfluidics, to separate cells into individual compartments, isolate RNA from the single cells, and generate cDNA for downstream genomic applications.

Downstream applications:

- RNA seq
- DNA seq
- PCR

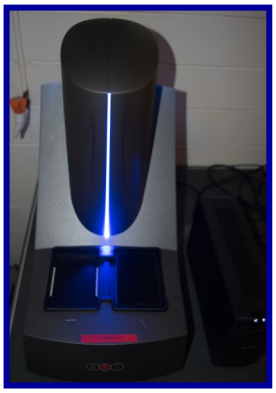
Format: 96 or 384 chambers per chip

MSD Multi-Array Platform

Operator: User

Applications:

- Detection of cytokines, cell signaling proteins
- Multiplexed assay design: (1-10 analytes/plate)
- Detection range: 1 – 10,000 pg/ml
- Sample volumes: 25 µl or less
- Assay Time: 4—6 hours depending on analytes being detected



Nanosight NS 300

Operator: User or Staff

Application:

- Determines the size and concentration of particles 10 nm to 1 microns in size
- Equipped with 4 lasers (405 nm, 488 nm, 532 and 642) to detect fluorescently labeled particles

Zetasizer Nano Z

Operator: User or Staff

Application:

Measures the zeta potential of particles in a solution using laser Doppler micro-electrophoresis

Upcoming Holidays & Events

November 2, 2020	Nanostring will present a seminar on their GeoMX Digital Spatial Profiling	TBA
November 3, 2020	Election Day Holiday	Facility Closed
November 25 – 27, 2020	Thanksgiving Holiday	Facility Closed
December 24 - 28, 2020	Winter Holiday	Facility Closed
December 31, 2020 - January 1, 2021	New Year's Holiday	Facility Closed

Note to Users

Please remember to acknowledge the support of the HSC Research Office and NIH grants that support the WVU Flow Cytometry & Single Cell Core in all your publications. The grant numbers are listed below:

TME CoBRE grant: P20GM121322

WVCTS grant: GM104942 important if you used the Miltenyi AutoMACS pro (installed 6/29/18)

WV InBRE grant: GM103434

Fortessa S10 grant: OD016165

NanoSight NS 300 use Stroke CoBRE grant GM109098 and WVCTS grant GM104942

ZetaSizer NanoZ use Stroke CoBRE grant GM109098 and WVCTS grant GM104942

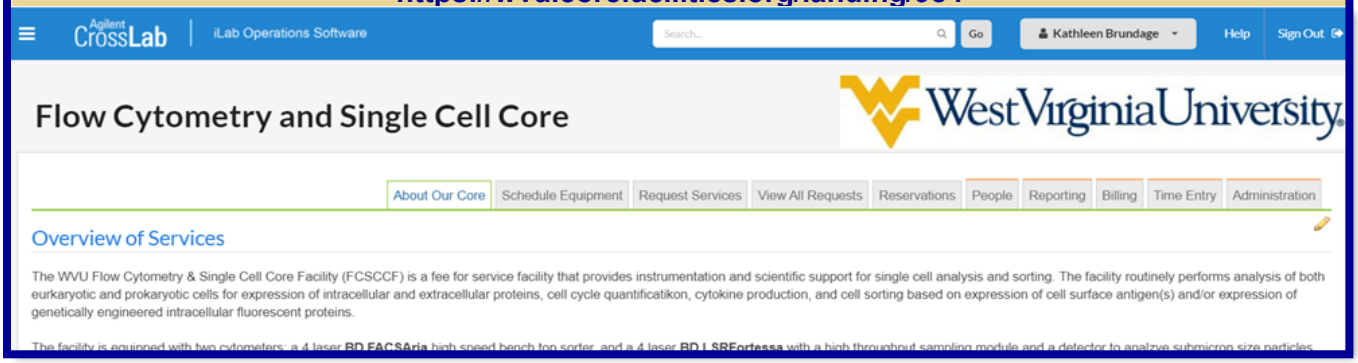
New User Guide

Hands-on training for LSRFortessa, C1 Single Cell Auto Prep System, NanoSight NS300 and Zetasizer Nano Z is **mandatory** for all new users and must be scheduled by consultation with the facility director.

Training will initiate with user's first experiment. Due to the complexity of the instruments and software, facility staff will fully assist with the acquisition of the first dataset and will continue with additional assistance on a "needs" basis until users are comfortable operating the instrument on their own. Sorting on the FACSAria is by facility staff only.

The facility uses iLAB scheduling/billing software from Agilent to manage the use of the facility's instrumentation. If you would like to use the instruments housed in the facility please use the URL shown below to register as a WVU User and to login to reserve an instrument.

https://wvu.corefacilities.org/landing/984



Fee Schedule

Instrument	Operator	For WVU & NIOSH Users	For Non-WVU Users
AutoMACS Pro	Facility Staff or User	$4.50 / separation	$6.85 / separation
C1 Single Cell Auto Prep System	Facility Staff	$210/plate	$320/plate
	User	No Cost	$115/plate
FACSAria III	Analysis: Facility Staff	$52.50/h	$80/h
	Analysis: User	$34.65/h	$53/h
	Sorting	$77.70/h	$120/h
	Sorting Setup	$19.43/sort	$30/sort
gentleMACS	Facility Staff or User	$10.50/sample	$16/sample
Guava easyCyte	Facility Staff	$52.50/h	$80/h
	User	$34.65/h	$53/h
LSRFortessa	Facility Staff	$52.50/h	$80/h
	User	$34.65/h	$53/h
MSD QuickPlex SQ120	Facility Staff or User	$10.50/h	$16/h
NanoSight NS300	Facility Staff	$61.00/h	$93/h
	User	$42.50/h	$65/h
Zetasizer Nano Z	Facility Staff	$25/sample + $52.50/h	$39/sample + $80/h
	User	$25/sample	$39/sample + $16/h