



# Complex analysis of metabolic status, intracellular pH, viscosity and cytoskeleton of human mesenchymal stem cells during differentiation by fluorescent microscopy and FLIM

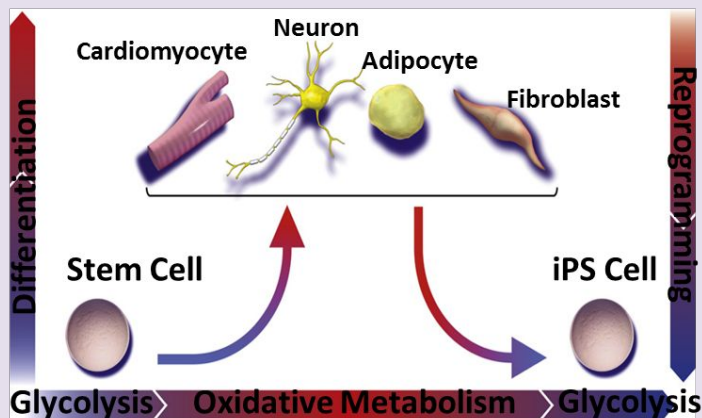
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<sup>1</sup>Nizhny Novgorod State Medical Academy, Nizhny Novgorod, Russia

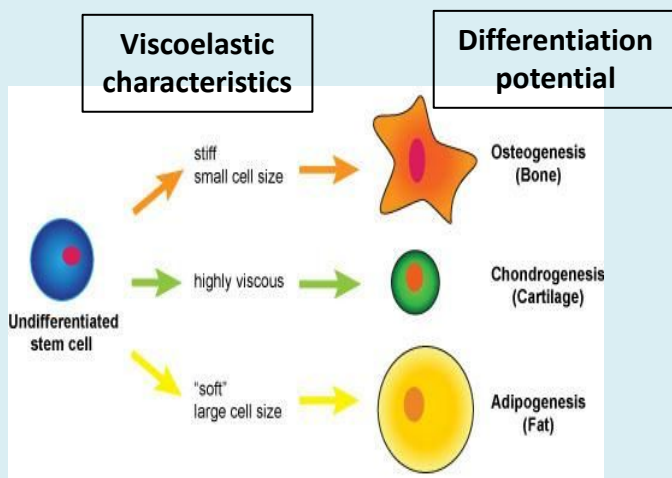
<sup>2</sup>Nizhny Novgorod State University, Nizhny Novgorod, Russia

# Functional-structural changes of MSCs during differentiation

## Metabolism

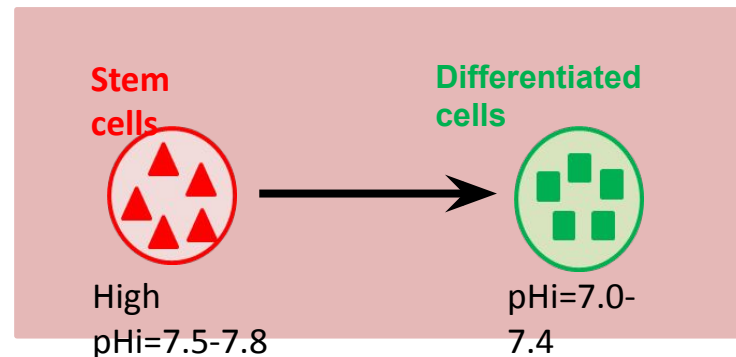


## Viscosity

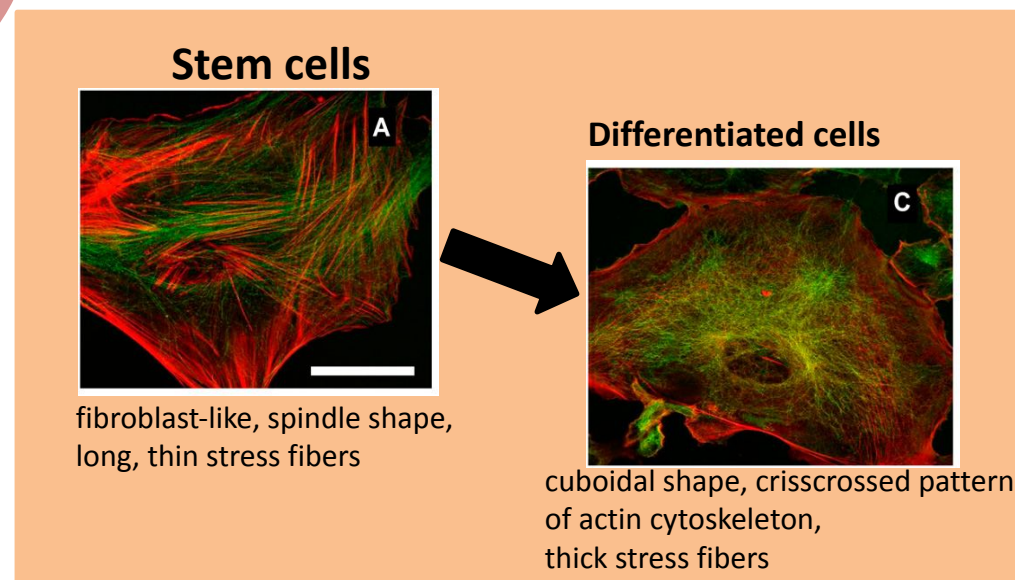


Mesenchymal Stem Cells

## pH



## Cytoskeleton



Effective control of MSCs differentiation - great challenge



Complex analysis is required!!!

# Methods of the stem cells morphology and physiology investigation



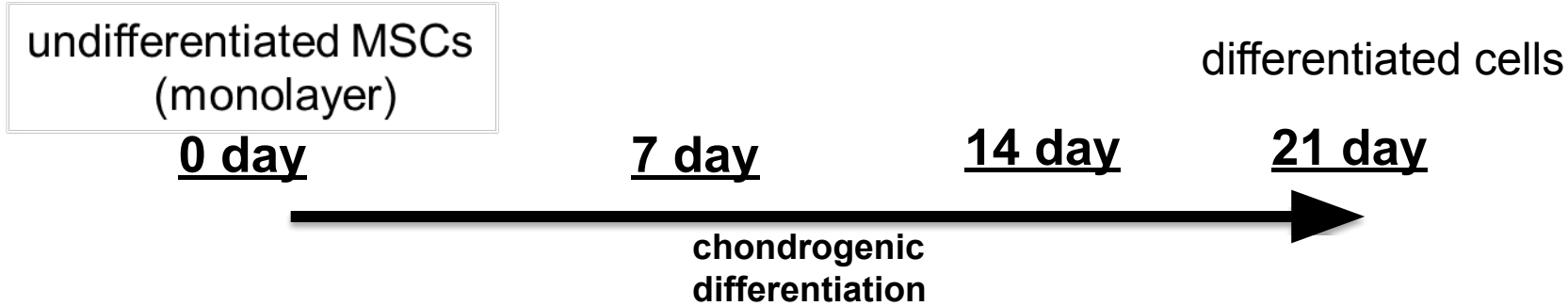
## Feature

## Method

Cell markers	<ul style="list-style-type: none"><li>• Flow cytometry</li><li>• Immunocytochemistry</li><li>• Magnetic-activated cell sorting</li></ul>
Genotype	<ul style="list-style-type: none"><li>• Polymerase chain reaction (PCR)</li></ul>
Differentiation potency	<ul style="list-style-type: none"><li>• Immunocytochemistry</li><li>• Fluorescence Microscopy + fluorescence dyes /protein</li><li>• Fluorescence Lifetime Imaging Microscopy (<b>FLIM</b>) +exo/endogenous markers</li><li>• Stochastic Optical Reconstruction Microscopy (<b>STORM</b>) +fluorescence dyes/protein</li></ul>

# Outline of the experiment

- **MSCs** – human mesenchymal stem cells bone marrow

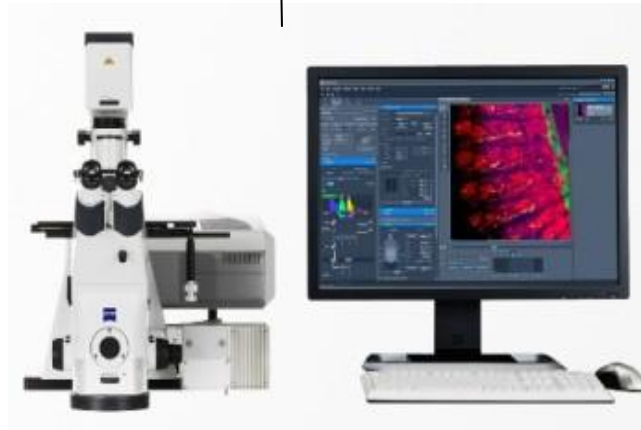


**Metabolism:** fluorescence microscopy and FLIM of NAD(P)H and FAD

**redox ratio FAD/NAD(P)H Lifetimes**

Nicotinamide adenine dinucleotide, **NADH:**  
excitation - 750 nm ,detection - 455-500 nm

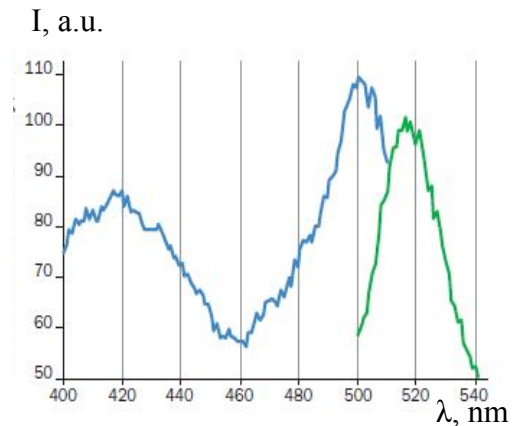
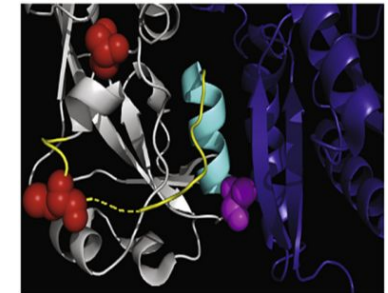
Flavine adenine dinucleotide, **FAD:**  
excitation - 900 nm , detection – 500-550 nm



**LSM 710** laser scanning confocal microscope (Carl Zeiss, Germany)  
**FLIM system** based on Simple Tau 152 TCSPC system (Becker & Hickl GmbH)

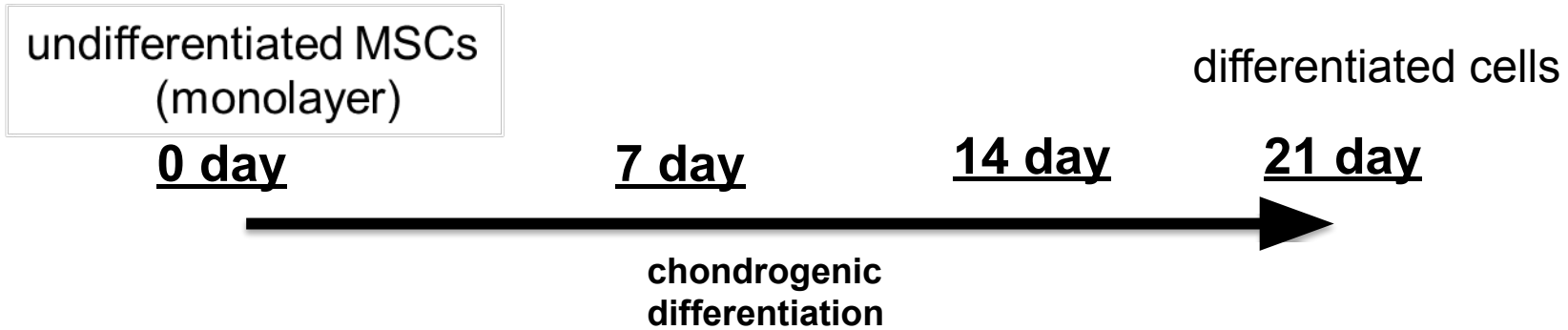
**pH:** fluorescence microscopy and SypHer-2

- YFP, monomer
- two peaks of fluorescence excitation (420 nm and 500 nm), peak emission 516 nm
- at **alkaline pH values**, the excitation peak at **420 nm decreases**, and at **500 nm - increases**, while for **acidic - on the contrary**

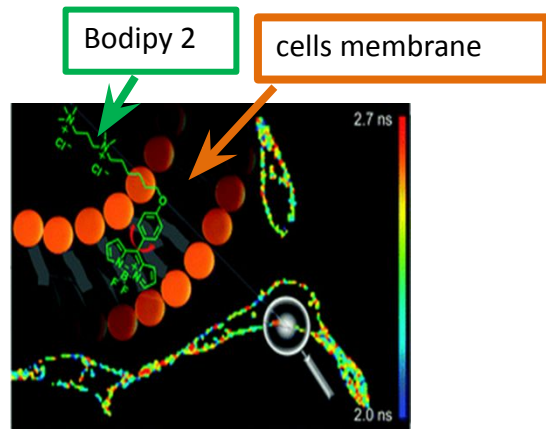


# Outline of the experiment

- **MSCs** – human mesenchymal stem cells bone marrow



## Viscosity: FLIM and Bodipy 2



ex = 800 nm,  
detection range = 409-660 nm



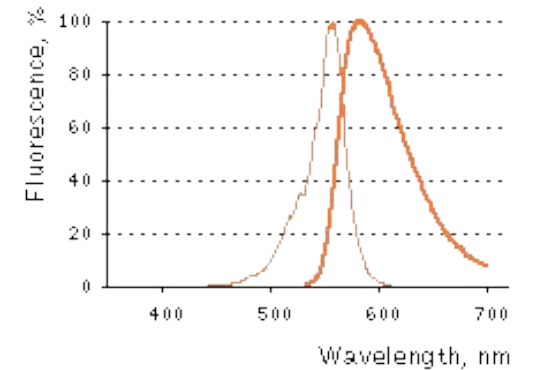
**LSM 710** laser scanning confocal microscope (Carl Zeiss, Germany)  
**FLIM system** based on Simple Tau 152 TCSPC system (Becker & Hickl GmbH)

## Cytoskeleton: STORM and TagRFP



**EclipseTi** (Nikon, Japan),  
module **N-STORM**, system PSF

### TagRFP

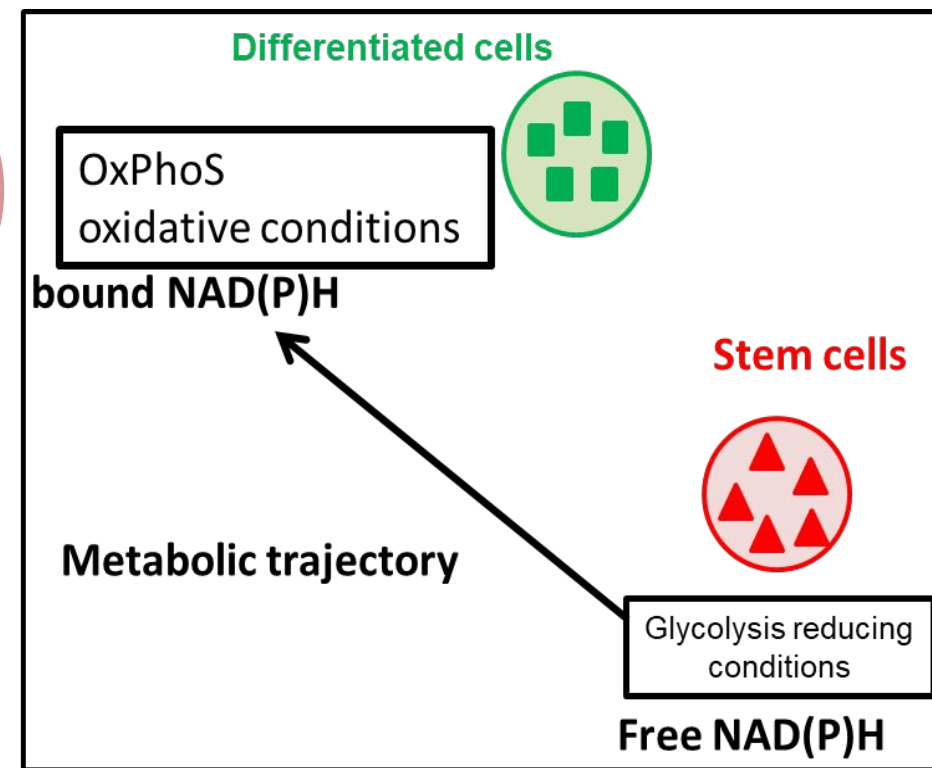
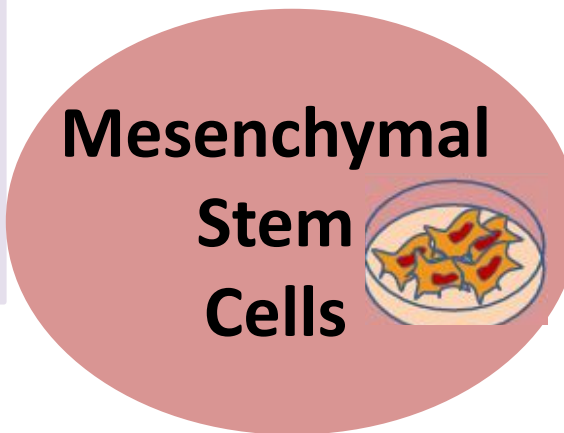
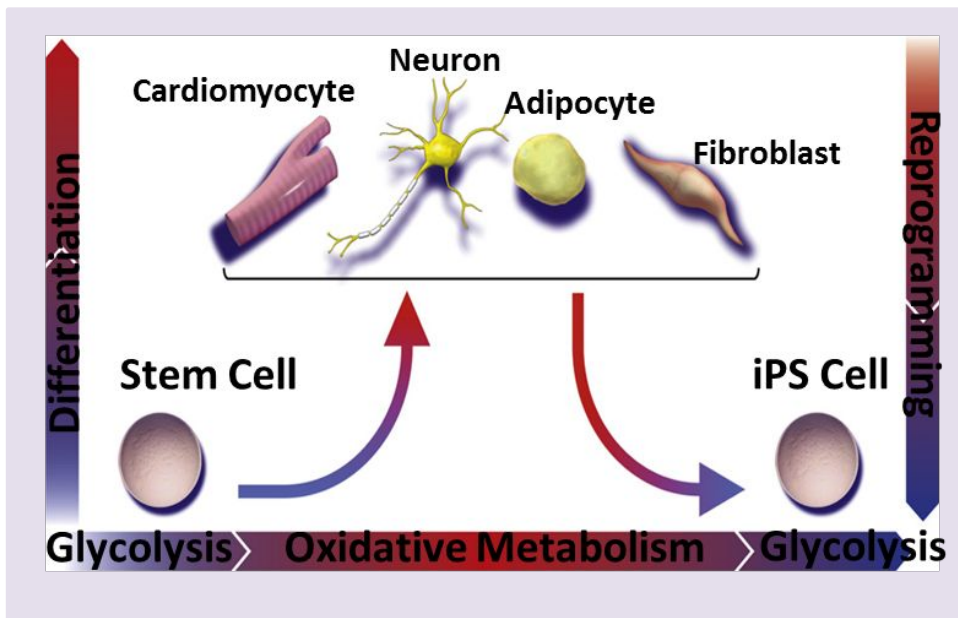


**em=550nm**  
**detection= 584nm**

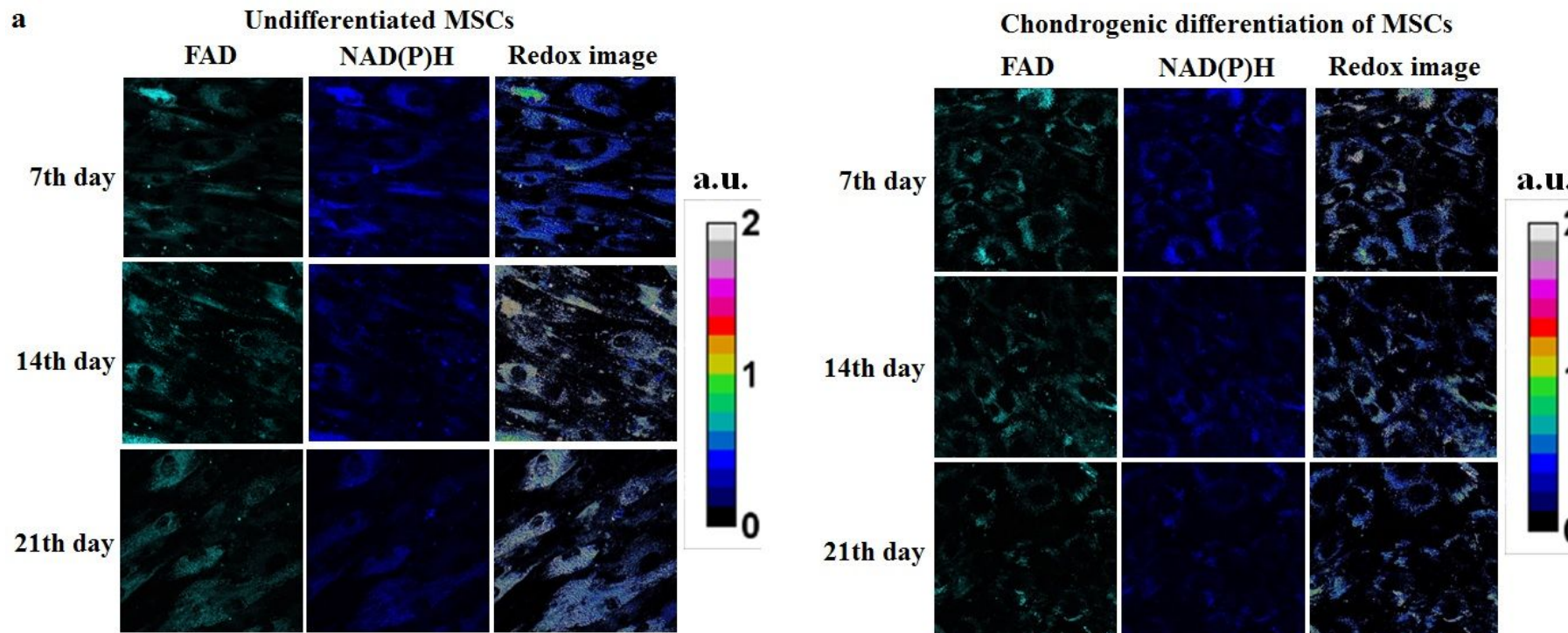


# Functional-structural changes of MSCs during differentiation

## Metabolism



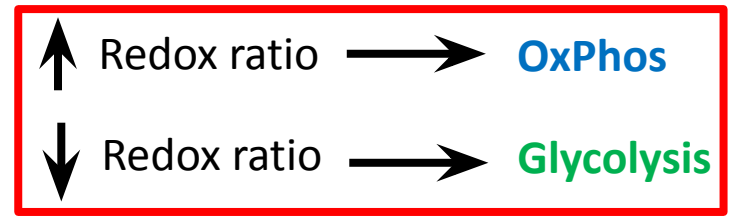
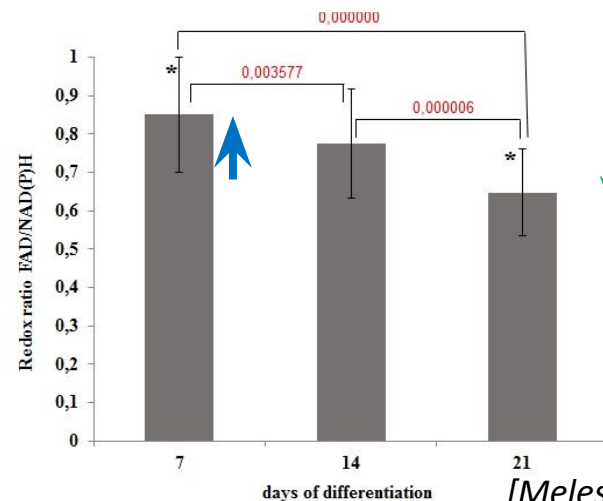
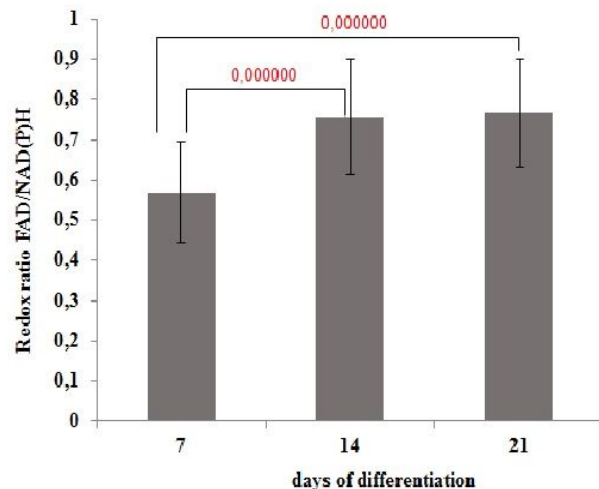
# Optical redox ratio of FAD/NAD(P)H changes during chondrogenic differentiation



**FAD:**  
excitation - 900 nm (5mW) ,  
detection - 500-550 nm

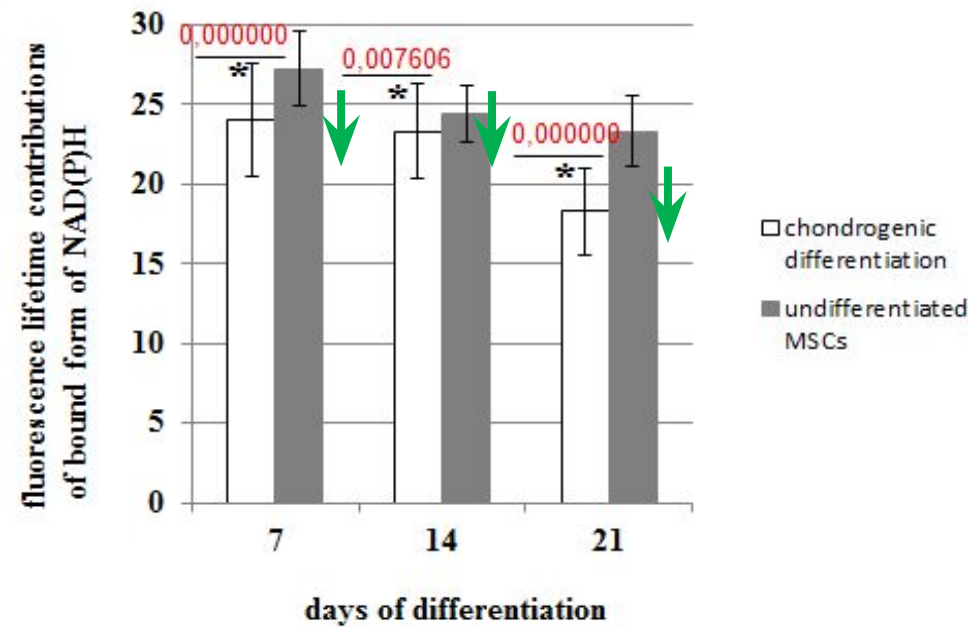
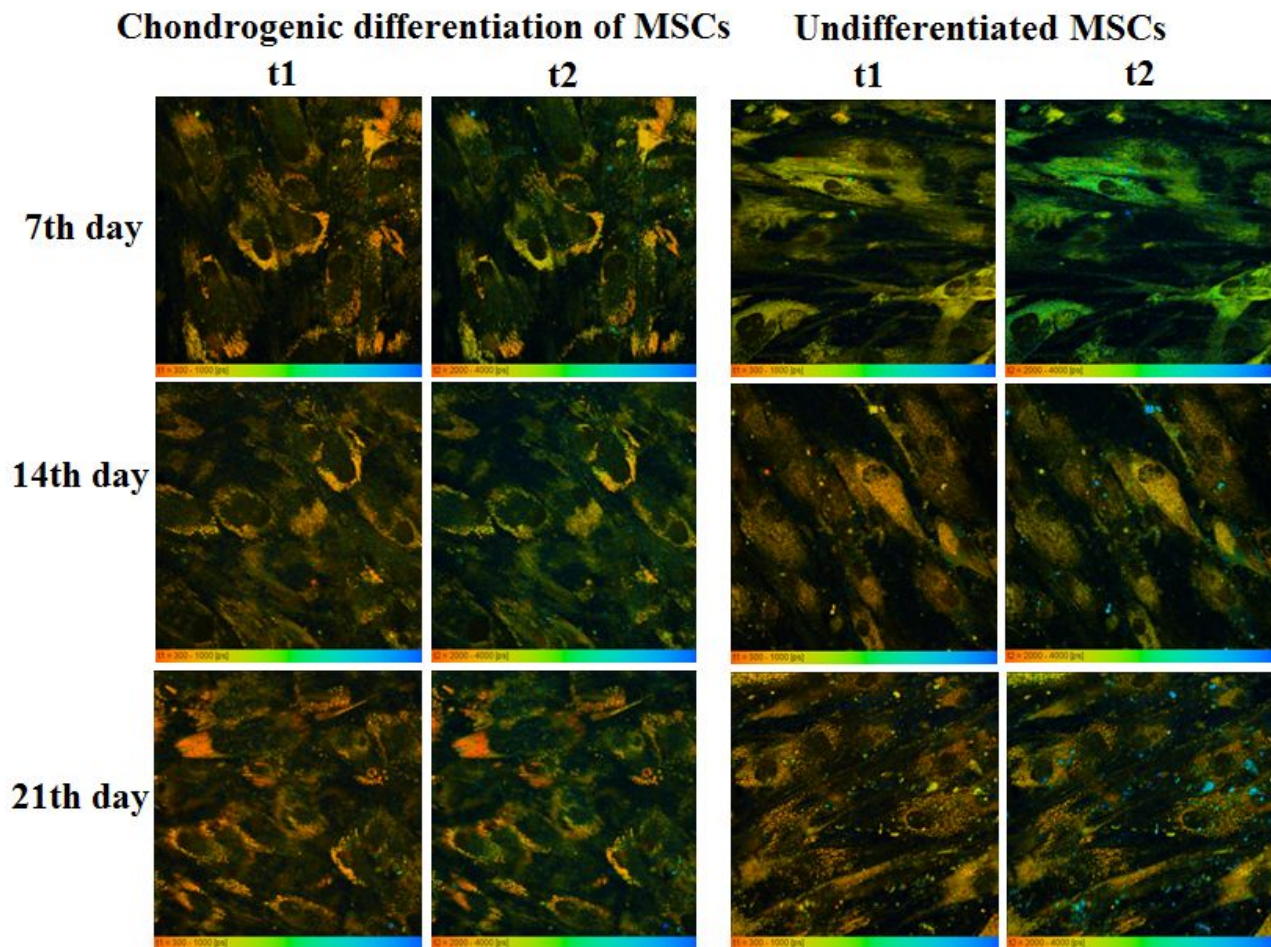
**NADH:**  
excitation - 750 nm (5 mW)  
detection - 455-500 nm

image size is 213 × 213 μm  
(1024 × 1024 pixels)





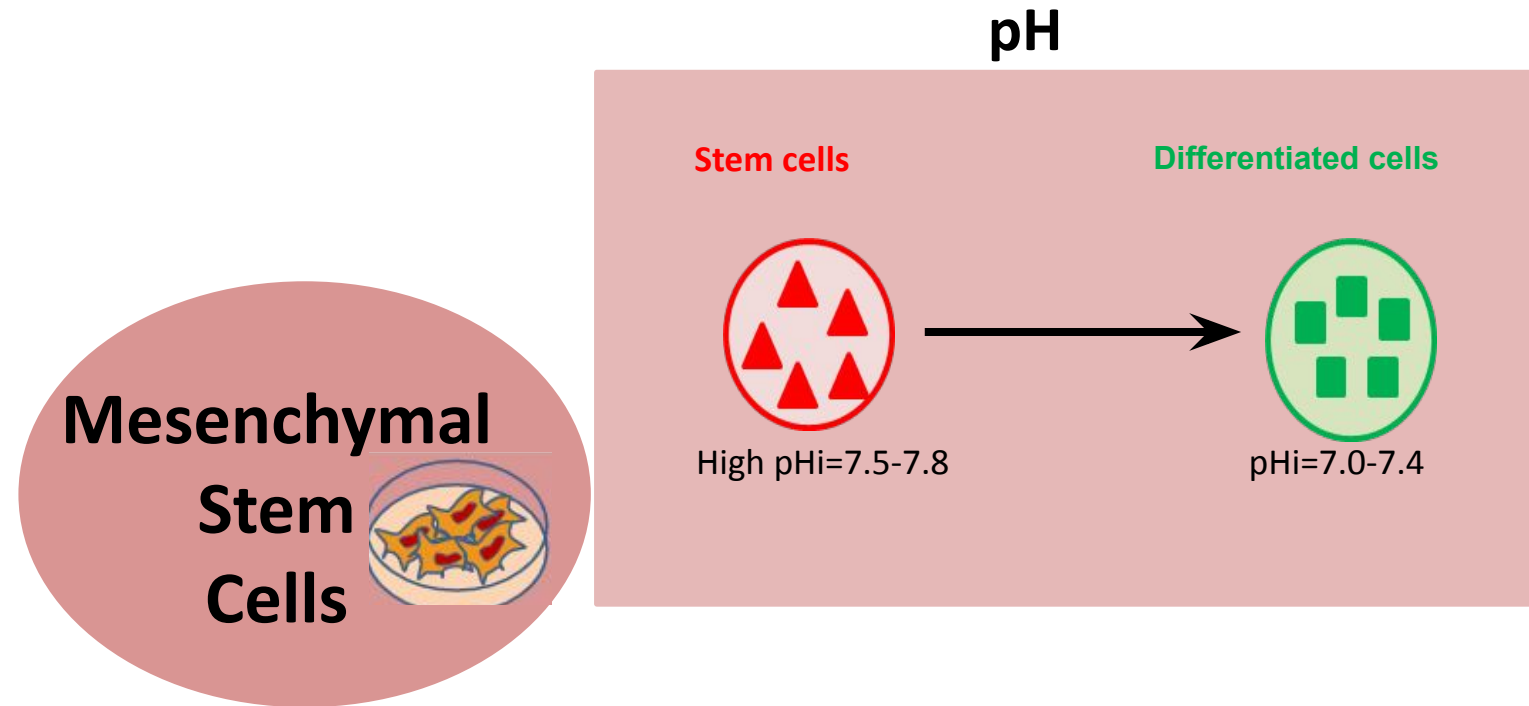
# Dynamic of bound NAD(P)H in MSCs during chondrogenic differentiation



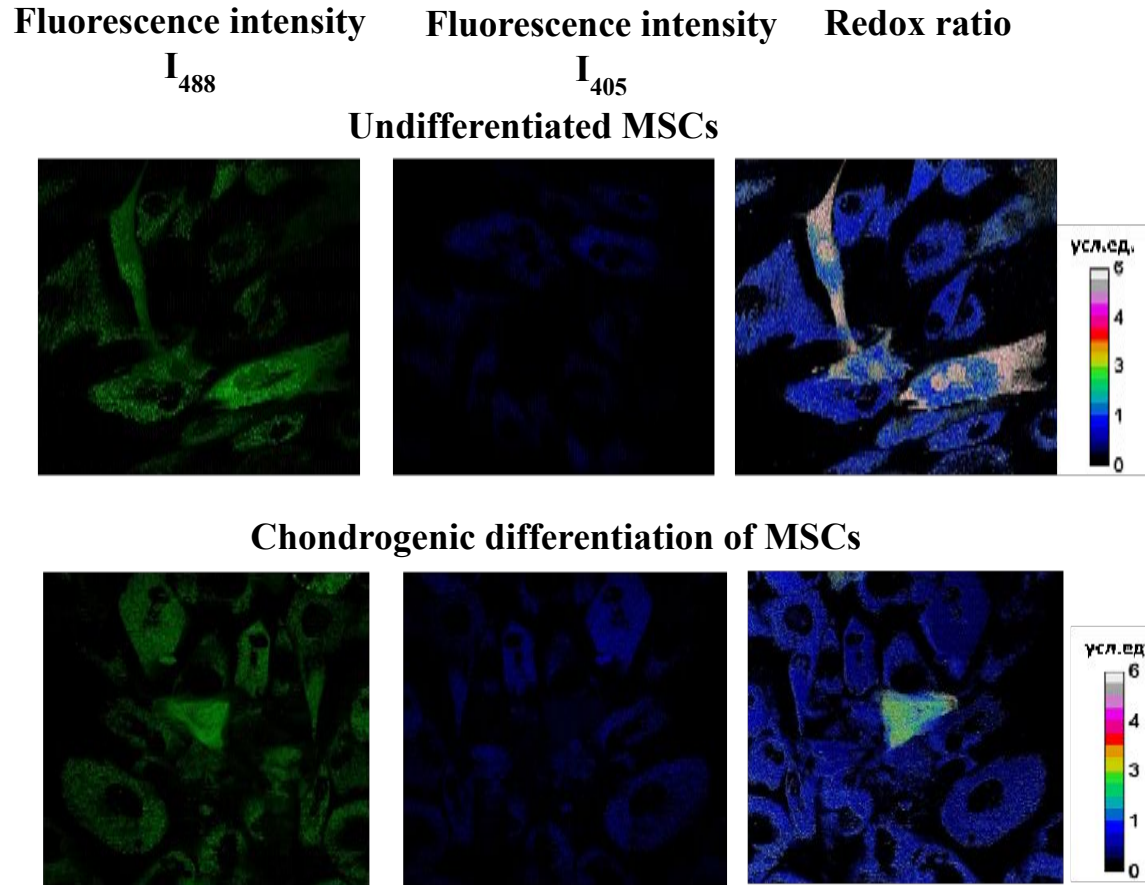
Pseudocolor-coded FLIM images of the free (t1) and protein-bound (t2) forms of NAD(P)H.

For NAD(P)H: excitation - 750 nm, detection - 455–500 nm. Field of view 213\*213µm (512\*512 pixels)

# Functional-structural changes of MSCs during differentiation

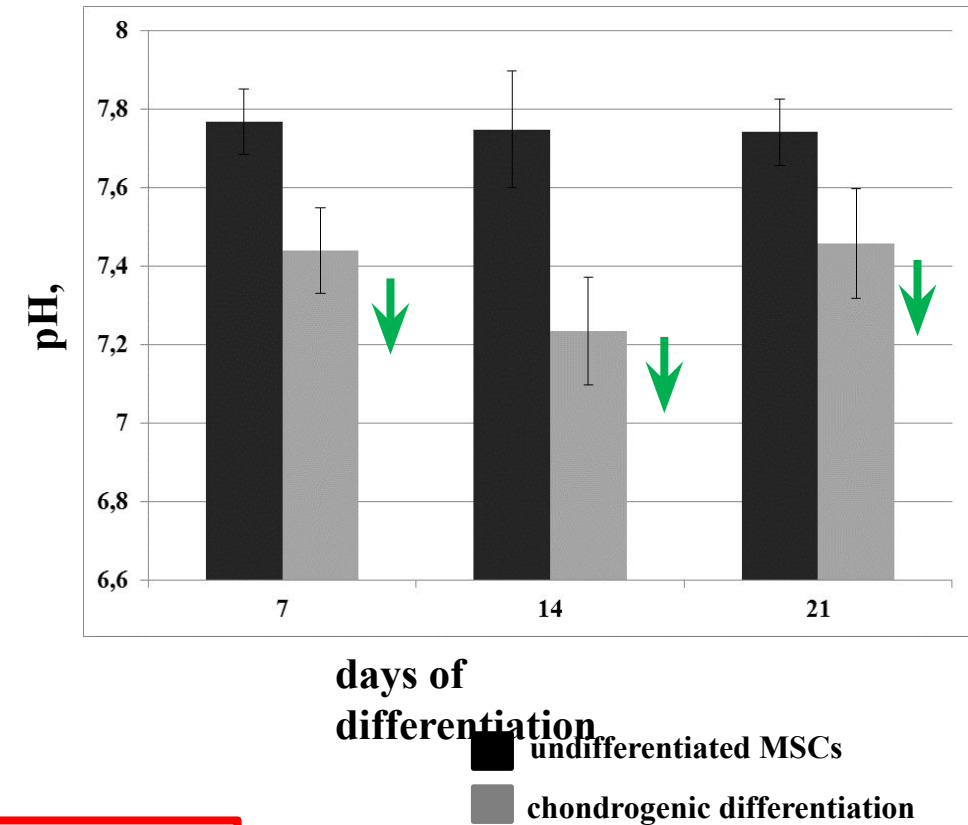


# Intracellular pH analysis in MSCs during differentiation by fluorescence microscopy and SypHer-2



ex = 405 nm and 488 nm, detection range = 500-550 nm

$$pH = I_{488}/I_{405} + 1,0219/0,2688$$

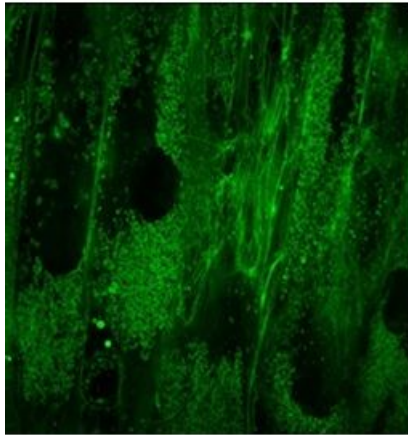


**bias to acidic pH values**

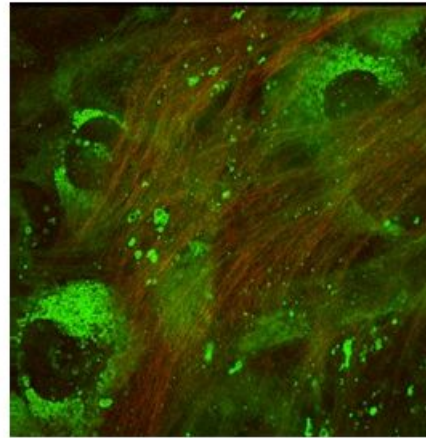


# Analysis of collagen formation during chondrogenic differentiation using SHG

Undifferentiated MSCs

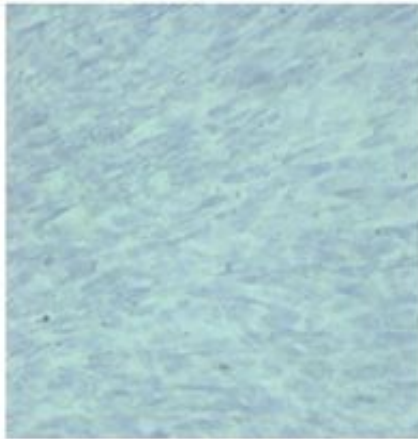


Chondrogenic differentiation of MSCs



green –  
cell autofluorescence

red- collagen fiber



Hematoxylin  
staining

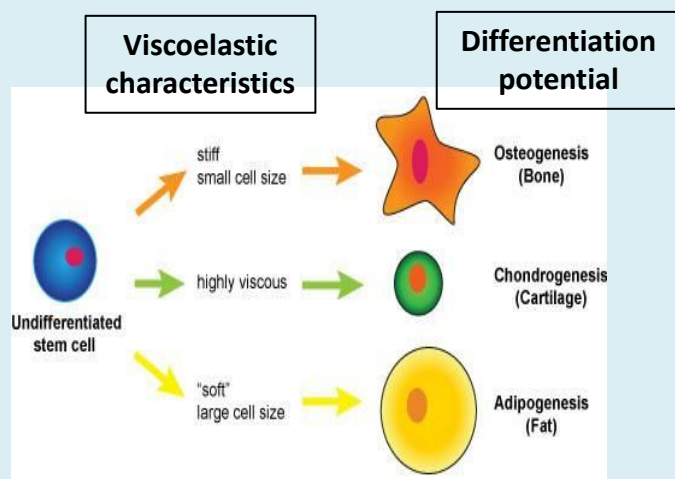


Alcian blue staining  
on acidic polysaccharides

SHG of collagen was excited at wavelength of 750 nm and detected in the range 373-387 nm  
the image size is 130×130 μm (512 × 512 pixels)

# Functional-structural changes of MSCs during differentiation

## Viscosity

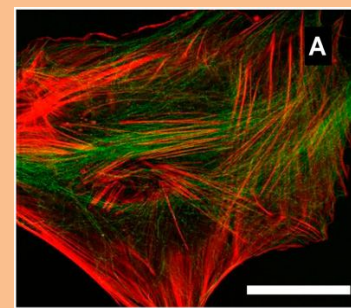


## Mesenchymal Stem Cells



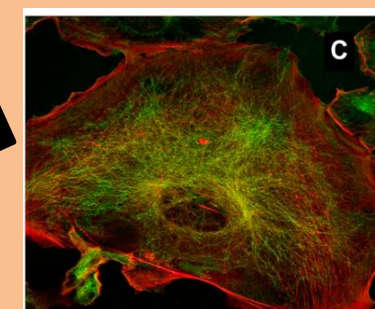
## Cytoskeleton

### Stem cells



fibroblast-like, spindle shape,  
long, thin stress fibers

### Differentiated cells



cuboidal shape, crisscrossed pattern  
of actin cytoskeleton,  
thick stress fibers

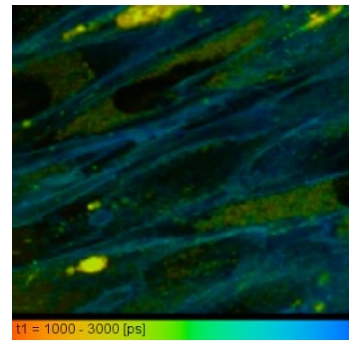
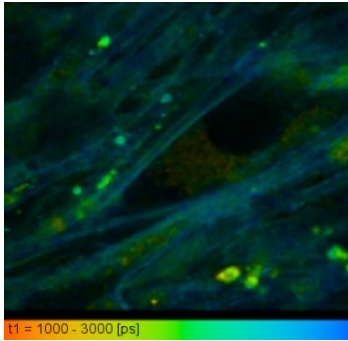


# MSCs viscosity analysis during differentiation using FLIM and Bodipy 2

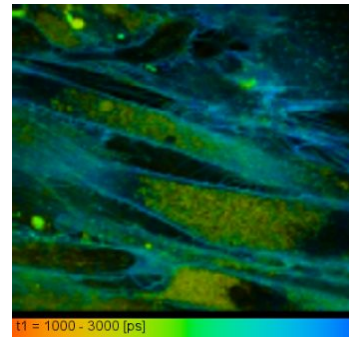
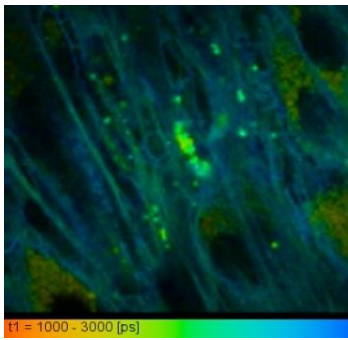
Chondrogenic differentiation of MSCs

Undifferentiated MSCs

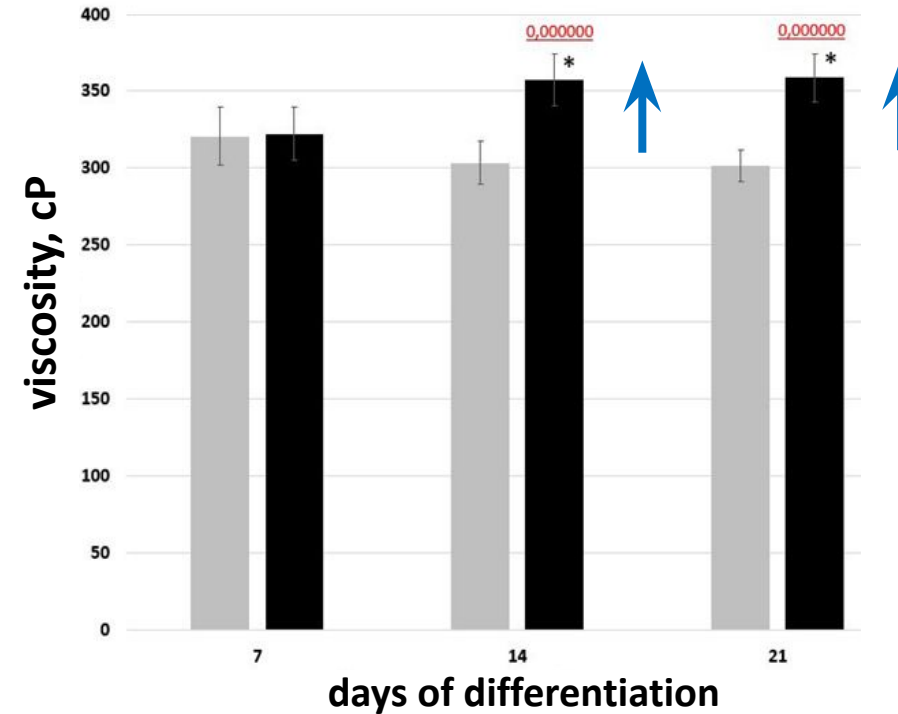
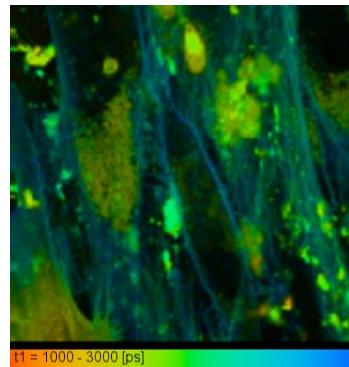
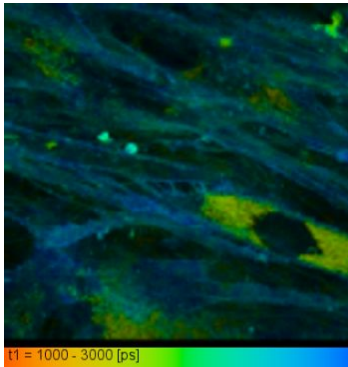
7 day



14 day



21 day

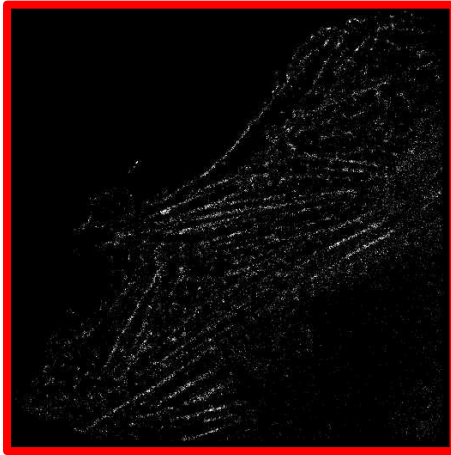


undifferentiated MSCs  
 chondrogenic differentiation

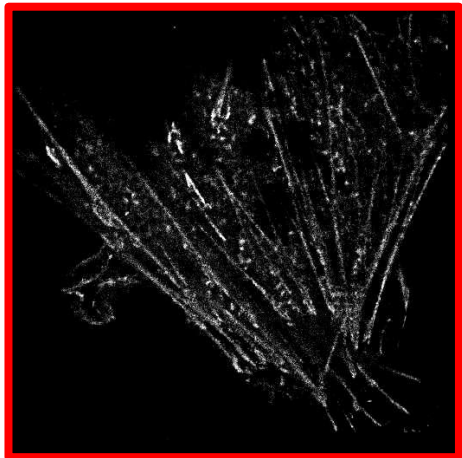
viscosity increase – cholesterol accumulation

# Analysis of cytoskeleton organization in MSCs during differentiation by STORM and TagRFP

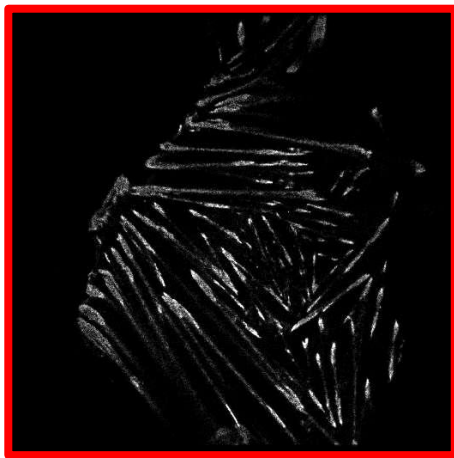
Undifferentiated MSCs



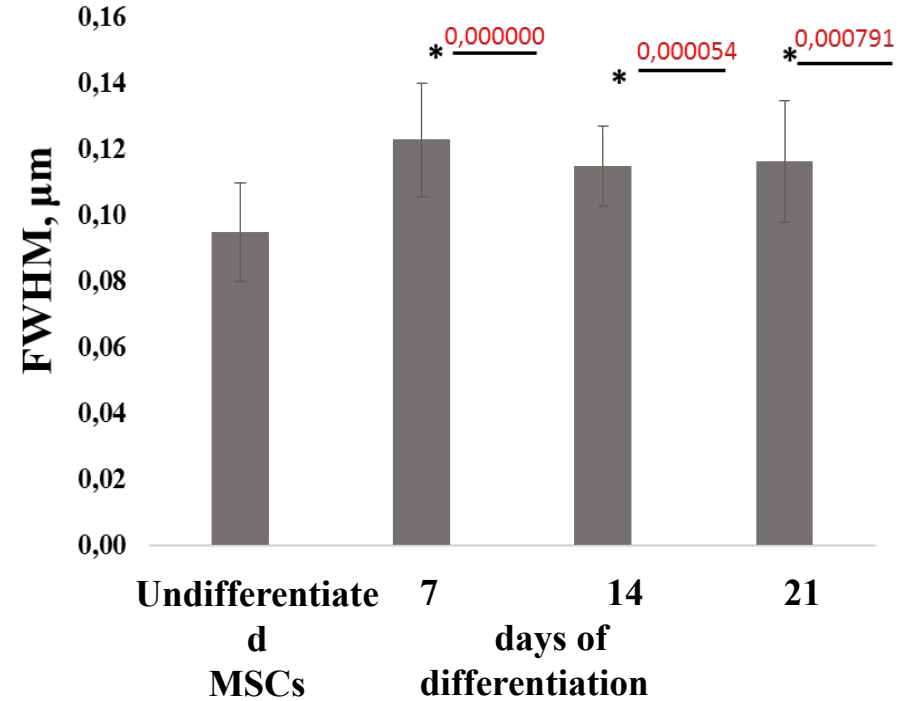
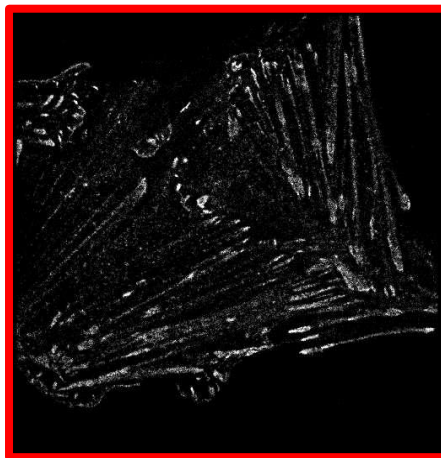
7 day



14 day



21 day



**Increase of actin fibers thickness**

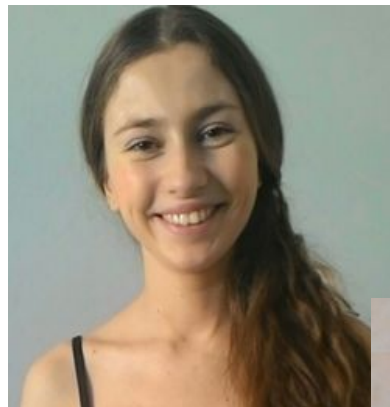
# take home message



- 1. Metabolic plasticity of MSCs** during chondrogenic differentiation: glycolysis – more glycolytic state
- 2. Intracellular pH**  
bias of pH values towards a more acidic pH
- 3. Membrane viscosity**  
viscosity increase – cholesterol accumulation
- 4. Cytoskeleton organization**  
undifferentiated MSCs having a fibroblast-like morphology, the actin fibers are represented by long, parallel fibrils extending through the cytoplasm of the cells. Chondrocytes have increased the thickness of end parts of actin fibers. In addition, chondrocytes have changed their orientation: actin fibrils crossed cells in different directions

# Acknowledgements

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V.V. Dudenkova



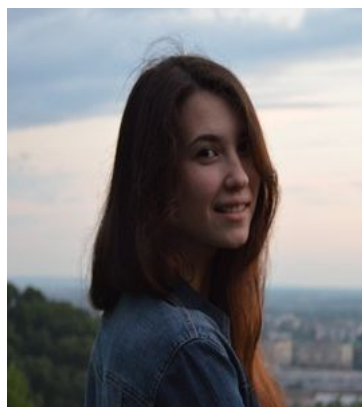
M.K. Kuimova



M.V. Shirmanova



E.V. Zagaynova



A.S. Bystrova



N.V. Klementieva



F.A. Kulagin



O. Furman





**Thank you for your attention!**