Study of the effect of laser pulse duration in the ultraviolet spectral range on fibroblasts

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Background

VITILIGO

UV THERAPY

The Ultraviolet (UV) light that penetrates our skin is made up of two types of radiation:

- UV-A rays
- UV-B rays

Skin Layers

UV-B
SOLAR RADIATION

UVA  UVB  UVC

400 nm  320 nm  280 nm  100 nm

Atmosphere, Ozone

Epidermis

Dermis

UVA biology effects
- Oxidative damage
- Abasic sites
- Base modifications
- Strand breaks

UVB biology effects
- Cell cycle changes
- Cyclo pyrimidine dimers
- Mutations
- 6-4 photoproducts

Collagen

FIBROBLASTS
Project aim

Study the effect of pulse duration and wavelength of laser light in the ultraviolet range of the spectrum on fibroblast cells.

Tasks:

1. To achieve a laser on the active medium LiLu0.7Y0.3F4:Ce3+,Yb3+ with the possibility of changing the pulse duration.

2. To study the survival of fibroblast cells when irradiated with laser light in the ultraviolet range of the spectrum with different duration and wavelength.
Experimental Setup:

Nd:YAG laser with a wavelength of 266 nm

Raman Shifter

High Reflector

Converging Lens

Output Coupler

Crystal LiLu$_{0.7}$Y$_{0.3}$F$_4$:Ce$^{3+}$,Yb$^{3+}$

300 nm

Converging Lens

Diaphragm

Pellin-Broca prism

High Reflector

Output Coupler
Experimental Setup:

\[ \Delta T = 10 \text{ нс} \]

\[ \Delta T = 1 \text{ нс} \]

Intensity, a.u.

Wavelength, nm

LiYLuF\({}_4\):Ce\(^{3+}\)Yb
Methodology

Dual channel power meter Ophir Pulsar-2

Four channel oscilloscope

Rohde & Schwarz

Spectrometer Solar laser systems S100

Cytometry

MTT assay
MTT assay

The MTT test is based on the reduction reaction of the yellow tetrazolium salt MTT by mitochondrial dehydrogenases of living cells to purple formazan crystals, which are insoluble in the aquatic environment of the cells. The amount of formazan formed is proportional to the number of viable cells in the well.
MTT results

Evaluation of changes in the viability of human skin fibroblasts under the effect of laser irradiation using the MTT test. HSF cells.

The survival of fibroblast cells is less when irradiated with UV light with a shorter pulse duration (1 ns) increasing in HSF cells viability after 24 of irradiation
MTT results

Evaluation of changes in the viability of human skin fibroblasts under the effect of laser irradiation using the MTT test. HSF cells.

The survival decreases with shorter wavelength.
# Results of Cytometry

<table>
<thead>
<tr>
<th>№ event</th>
<th>percent of events</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.4%</td>
<td>DiOC6+PI- (alive)</td>
</tr>
<tr>
<td>II</td>
<td>12.6%</td>
<td>DiOC6-PI- (with damaged mitochondria)</td>
</tr>
<tr>
<td>III</td>
<td>6.2%</td>
<td>DiOC6-PI+ (dead, late apoptosis)</td>
</tr>
<tr>
<td>IV</td>
<td>80.8%</td>
<td>DiOC6+PI+ (perforated cytoplasmic membrane)</td>
</tr>
</tbody>
</table>

DiOC6(3) : 3,3’-dihexyloxacarbocyanine iodide.  
PI : propidium iodide.
Conclusion

1. Laser generation on the LiLu0.7Y0.3F4:Ce3+,Yb3+ active medium in the pulsed mode with wavelength tuning from 305 to 335 nm, as well as with different pulse durations, has been achieved.

2. The viability of human skin cells under the action of laser UV radiation was assessed.

- It has been shown that the survival of fibroblast cells is less when irradiated with UV light with a shorter pulse duration (1 ns)

- It has also been shown that survival decreases as the wavelength of UV laser light is shortened.

- It has been established by flow cytometry that as a result of UV laser irradiation of fibroblasts, the cytoplasmic membrane is damaged in 80% of cases.
Acknowledgments

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Supervisor

Dr. Alexey Nizamutdinov
References


• [7] Ultra-short pulses UV lasing in multifunctional Ce:LiY0.3Lu0.7F4 active medium, Farukhshin, I.I., Nizamutdinov, A.S., Korableva, S.L., Semashko, V.V., Optical Materials Express, 2016, 6(4), стр. 1131–1137
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