Femtosecond laser applied to biophotonics

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short pulse duration $\rightarrow$ high intensity

(even at low energy)
how short is a femtosecond pulse?

1 fs = 10^{-15} s
Introduction

How short is a femtosecond pulse?
Ti:Sapphire lasers

100 fs  50 fs  20 fs

Very intense light

Laser intensities $\sim 100$ GW/cm$^2$

$1 \times 10^{11}$W/cm$^2$

Laser pointer: 1 mW/cm$^2$ ($1 \times 10^{-3}$ W/cm$^2$)
introduction

Ti:Sapphire lasers

100 fs    50 fs    20 fs

Very intense light

Nonlinear Optical Phenomena
Light matter interaction

Semiclassical treatment

electron on a spring

oscillation frequency

$$\omega_0 = \sqrt{\frac{k}{m_e}}$$
Linear optical processes

\[ E_{\text{radiation}} \ll E_{\text{interatomic}} \]

Induced polarization

\[ P = \chi E \]

linear response

absorption \[ \alpha = \alpha(\lambda) \]

refraction \[ n = n(\lambda) \]

independent of the light intensity
Nonlinear optical processes

How high should be the light intensity?

\[ E_{\text{rad.}} \sim E_{\text{inter.}} \]
Nonlinear Optics

Inter-atomic electric field

- $e = 1.6 \times 10^{-19}$ C
- $r \sim 4 \text{ Å}$
- $E \sim 1 \times 10^{10}$ V/m

$E_0 = 4 \times 10^6$ V/m

cw laser

- $P = 20$ W
- $w_0 = 20$ μm

$I = \frac{2P}{\pi w_0^2}$

$I = 3 \times 10^{10}$ W/m²
Nonlinear Optics

Inter-atomic electric field

\[ e = 1.6 \times 10^{-19} \text{ C} \]

\[ r \sim 4 \text{ Å} \]

\[ E \sim 1 \times 10^{10} \text{ V/m} \]

pulsed laser

\[ I = 10 \text{ GW/cm}^2 = 10 \times 10^{13} \text{ W/m}^2 \]

\[ I = \frac{1}{2} c \varepsilon_0 E_0^2 \]

\[ E_0 = 1 \times 10^8 \text{ V/m} \]
Nonlinear Optics

Anharmonic oscillator

-high light intensity

\[ E_{\text{rad.}} \sim E_{\text{inter.}} \]

Nonlinear polarization response

\[ P = \chi^{(1)}E + \chi^{(2)}E^2 + \chi^{(3)}E^3 + \ldots \]
Second harmonic generation

Second order processes \( \chi^{(2)} \)

\[ \omega \rightarrow \chi^{(2)} \rightarrow 2\omega \]
Two-photon absorption

Third order processes $\chi^{(3)}$

$\alpha = \alpha_0 + \beta I$
Multi-photon absorption

\[ \chi^{(3)}, \chi^{(5)}, \chi^{(7)}, \ldots \]

\[ \alpha = \alpha_0 + \beta I + \alpha_3 I^2 + \alpha_4 I^3 + \alpha_5 I^4 + \ldots \]
Nonlinear interaction provides spatial confinement of the excitation

\[ \alpha = \alpha_0 \quad \alpha = \alpha_0 + \beta I \]
• microstructuring
• microfabrication
fs-laser microstructuring

photon energy < bandgap

nonlinear interaction
nonlinear interaction

\[ E_{\text{gap}} \]

\[ E_f = h\nu \]
fs-laser microstructuring

nonlinear interaction

\[ E_{\text{gap}} \]

\[ E_f = h\nu \]

multiphoton absorption
fs-laser microstructuring

amplified laser

oscillator

1 ms

repetitive

508x223

12 ns

cumulative

Micromachining the sample’s Volume or Surface
microstructuring surfaces

[Diagram showing components of a laser setup including CCD, lens, mirror, objective (NA 0.65), fs-laser, and sample.]
microstructuring surfaces
fs-laser micromachining

Latex - natural rubber of the clones: GT 1

Production of latex-based scaffolds for cellular growth
Microstructuring Latex

influence of pulse energy in the micromachining of Latex

1.85 µJ  1.21 µJ  0.97 µJ  0.90 µJ  0.74 µJ  0.61 µJ
Microstructuring Latex

High resolution and small collateral damage

\begin{itemize}
\item $E = 1.21 \, \mu J$
\item $E = 0.61 \, \mu J$
\end{itemize}
Microstructuring Latex

Some of the surface patterning produced on latex.

no carbonization of the latex has been observed
Relatively large areas can be produced with this method
Scaffolds for neuron growth

fs-laser microfabrication to produce scaffolds for neuron growth
Scaffolds for neuron growth
Scaffolds for neuron growth

Neuron growth platforms need very specific biopolymers

approach

1 - Microstructure glass surface
2 – Stamping with PDMS
Fabrication of the molds

1. fs-micromachining y
2. fs-micromachining x
3. Glass substrate
4. Peel away
5. "Master"
6. Pour on PDMS and cure
Fabrication of the molds

examples of micromachined surfaces in glass
Microfabrication

Novel concept:

build microstructures using fs-laser and nonlinear optical processes
Two-photon polymerization

Monomer + Photoinitiator $\rightarrow$ Polymer

Photoinitiator is excited by \textit{two-photon absorption}

$$R_{2PA} \propto I^2$$

The polymerization is confined to the focal volume.

High spatial resolution
Two-photon polymerization setup

Ti:sapphire laser oscillator
- 130 fs
- 800 nm
- 76 MHz
- 20 mW

Objective
- 40 x
- 0.65 NA
Two-photon polymerization
Resin preparation

Monomers

Monomer A

\[
\begin{align*}
\text{CH}_2\text{CH}_2\text{C}(\text{O}\text{CH}_2\text{CH}_2\text{O}\text{CH}_2\text{CH}_2\text{O})\text{CH}_2\text{C} = \text{CH}_3 \\
\text{CH}_2\text{CH}_2\text{C}(\text{O}\text{CH}_2\text{CH}_2\text{O}\text{CH}_2\text{CH}_2\text{O})\text{CH}_2\text{C} = \text{CH}_3
\end{align*}
\]

reduces the shrinkage upon polymerization

Monomer B

\[
\begin{align*}
\text{CH}_2\text{CH}_2\text{C}(\text{O}\text{CH}_2\text{CH}_2\text{O}\text{CH}_2\text{CH}_2\text{O})\text{CH}_2\text{C} = \text{CH}_3 \\
\text{CH}_2\text{CH}_2\text{C}(\text{O}\text{CH}_2\text{CH}_2\text{O}\text{CH}_2\text{CH}_2\text{O})\text{CH}_2\text{C} = \text{CH}_3
\end{align*}
\]
gives hardness to the polymeric structure

Photoinitiator

Lucirin TPO-L

\[
\begin{align*}
\text{H}_3\text{C} \quad \text{O} \\
\text{CH}_3 \quad \text{O} \\
\text{H}_3\text{C} \quad \text{O} \\
\text{CH}_3
\end{align*}
\]

Two-photon polymerization

Ti:sapphire laser, 800 nm wavelength, 130 fs pulses. The laser beam is focused through an objective lens, polymerizing the monomer into a 30 µm x 30 µm x 12 µm cube on a glass substrate.
After the fabrication, the sample is immersed in ethanol to wash away any unsolidified resin and then dried.
Two-photon polymerization

Microstructures fabricated by two-photon polymerization
Stem cell differentiation

fabrication of specific 3D scaffolds for stem cell growth and differentiation
Stem cell scaffolds

20 μm

20 μm
Stem cell scaffolds
Stem cell differentiation

Adhesion
Stem cell differentiation

Proliferation
Stem cell differentiation

Differentiation
Microstructures with active compounds

Optical active dye

Active Polymer
Doping microstructures

Fabrication of microstructures with special topological and chemical design for bio-relates applications
Doping microstructures

- microstructures containing biopolymer - chitosan
Microstructures containing Rhodamine

Rhodamine 6G

- High luminescence
- Used as dye laser gain medium
Microstructure containing Rhodamine
Microstructure containing Rhodamine
Microstructure containing Rhodamine
Microstructure containing Rhodamine

Diagram showing a setup with an Ar⁺ laser and white light, focusing on a sample, and capturing fluorescence with a CCD camera.
Microstructure containing Rhodamine

Diagram showing a setup with an Ar+ laser, white light, a sample, a fluorescence detector, and a spectrum graph.
Microstructure containing Rhodamine

The diagram shows the fluorescence intensity (arb. units) as a function of wavelength (nm). The intensity peaks around 590 nm, with a peak intensity of approximately 0.8.
Microstructure containing Rhodamine

fabrication of array of doped microstructures
Microstructure containing Rhodamine

Fluorescent confocal microscopy

planes separated by 6 μm
Guiding bacterial growth in a micro-environment

microfabrication of multi-doped microstructures
Guiding bacterial growth in a micro-environment

double doped microstructure

Induce cell growth in distinct regions
Double doped microstructures fabrications

microstructure containing Fluorescein and Rhodamine
Double doped microstructures fabrications
Viability of the Lactobacillus in the resin

day 0  
day 3  
day 7
Viability of the Lactobacillus in the resin

day 0
Viability of the Lactobacillus in the resin

day 3
fs-laser spectroscopy of bio-materials

- multi-photon absorption
- nonlinear refraction
- excited state absorption processes
- dynamics of ultrashort optical processes
fs-laser spectroscopy of bio-materials

all-trans retinal

all-trans retinal cytochrome c

all-trans β-carotene

trans β-apo-8´carotenal

Poster: Marcelo G. Vivas
Summary
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