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Luís Manuel Couto Oliveira •  
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# The Optical Clearing Method

A New Tool for Clinical Practice and  
Biomedical Engineering

 Springer

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# Preface

The optical immersion clearing method allows one to increase tissue transparency through the reduction of light scattering. The method has considerable potential that has captured researcher's interest in the last two decades and produced an increasing number of publications in the past few years. In recent years, this technique has been applied in various *in vitro*, *ex vivo*, and *in vivo* biological materials, allowing, among others, the evaluation of the magnitude of the transparency effects, evaluation of mobile water in tissues, estimation of the diffusion properties of agents in biological tissues and liquids, discrimination between normal and pathological tissues, and characterization of the mechanisms involved in the optical clearing process. Considering that more than 10 years have passed since the first monograph has been published about the optical clearing method, we decided to write the present monograph to review and discuss some novel aspects of this fast growing research field.

This book covers the most recent discoveries in the field of optical clearing of biological tissues, the data that can be acquired from experimental studies, and its application in many fields of clinical practice or biomedical engineering. Chapter 1 describes the problem of tissue scattering and the limitations to the use of light-based methods in clinical practice. Chapter 2 describes various methods to increase tissue transparency and presents an historical description of the optical immersion clearing method. Some typical optical clearing agents and their optical properties are presented in Chap. 3, while the mechanisms of optical clearing are described in Chap. 4. The measurements that can be performed from *ex vivo* or *in vivo* tissues during optical clearing treatments are described in Chap. 5, and the data that can be retrieved from those measurements is described in Chap. 6.

Another very important field is tissue imaging. The most recent developments in tissue clearing that are directly connected to tissue imaging are presented in Chap. 7. The particular application of tissue and organ preservation is intimately connected with the optical clearing method, since most optical clearing agents are also used as

cryoprotective agents. Data that can be acquired during treatments, and are useful for other areas of biomedical engineering, such as tissue preservation, are presented in Chap. 8. Chapter 9 discusses the future of optical clearing in clinical practice, tissue and organ preservation, food industry, and other fields of biomedical engineering.

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We express our gratitude to our families for their indispensable support, understanding, and patience during the writing of this book.

# Abbreviations

C <sub>e</sub> 3D	Clearing-enhanced 3D
CEES	Chloroethyl ethyl sulfide
CLSM	Confocal light-sheet microscope
CM	Confocal microscopy
CPA	Cryoprotective agent
CPE	Chemical penetration enhancers
CSF	Cerebral spinal fluid
CT	Computed tomography
CUBIC	Clear unobstructed brain imaging cocktails
DMSO	Dimethyl sulfoxide
ECi	Ethyl cinnamate
e-Cig	Electronic cigarette
EG	Ethyleneglycol
ETC	Electrophoretic tissue clearing
FPT	Fructose + PEG-400 + thiazone solution
Hct	Hematocrit
HF	Hydrofluoric acid
HO	Hyperoxia exposure
IAD	Inverse adding doubling
IMC	Inverse Monte Carlo
ISF	Interstitial fluid
LSCI	Laser speckle contrast imaging
LSFEM	Light-sheet fluorescence expansion microscopy
MPT	Multiphoton tomography
MRI	Magnetic resonance imaging
NA	Numerical aperture
NaCl	Sodium chloride
NADPH	Nicotinamide adenine dinucleotide phosphate
NIR	Near infrared
OC	Optical clearing



OCA	Optical clearing agent
OCT	Optical coherence tomography
ODD	Optical detection depth
P	Permeability rate
PBS	Phosphate buffered saline
PEG	Polyethylene glycol
PEGASOS	Polyethylene glycol associated solvent system
PET	Positron emission tomography
PG	Propylene glycol
PMT	Photomultiplier tube
RI	Refractive index
RIMS	Refractive index matching solution
RPE	Retinal pigment epithelium
RSDL	Reactive skin decontamination lotion
SC	Stratum corneum
SD	Standard deviation
SDS	Sodium dodecyl sulfate
SeeDB	See deep brain
SHG	Second harmonic generation
SLED	Super-luminescent light emitting diode
SLS	Sodium lauryl sulfate
SPIM	Selective plane illumination microscopy
TDE	2,2'-thiodiethanol
TELS	Tissue engineered liver slices
UV	Ultraviolet
VF	Volume fraction

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