

Laser-ultrasonic delivery of agents into articular cartilage

Heikki J. Nieminen, Gonçalo Barreto, Mikko A. Finnilä, Alejandro García-Pérez, Ari Salmi, Sanjeev Ranjan, Kari K. Eklund, Kenneth P.H. Pritzker, Simo Saarakkala, Edward Hægström

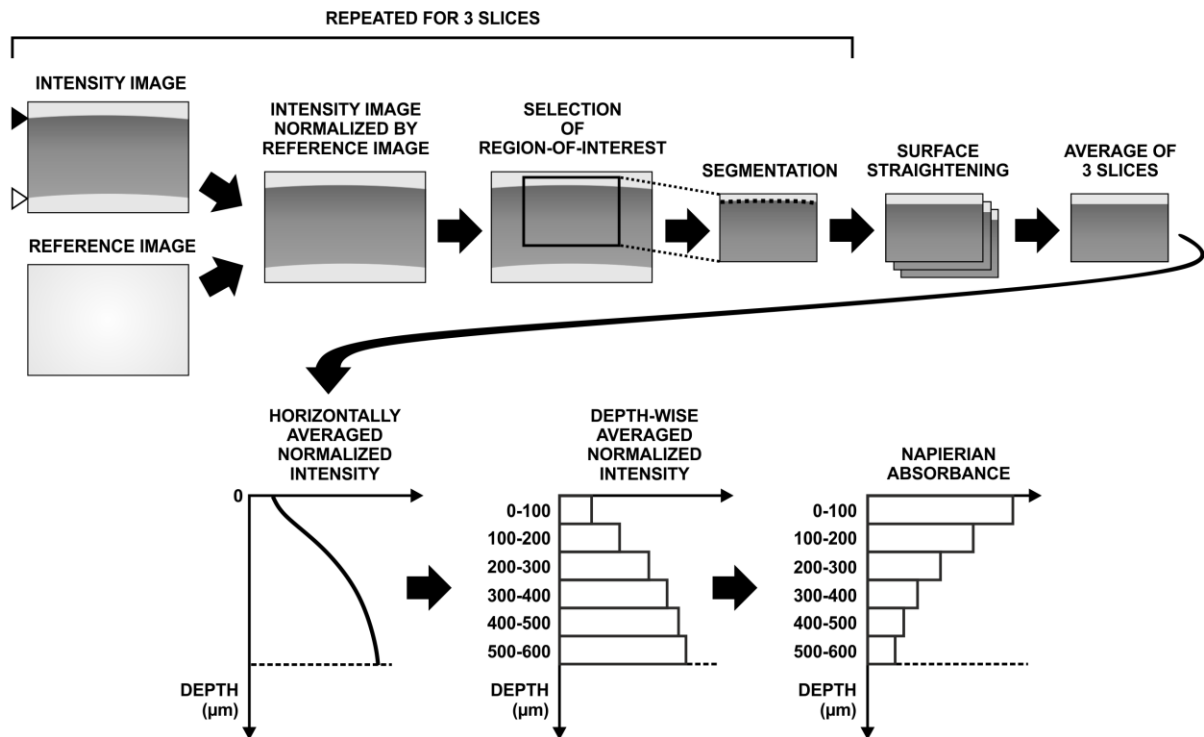


Figure A1: The delivery of methylene blue (MB) was detected from light microscope images acquired in through transmission mode (light wavelength $\lambda = 657 \text{ nm}$). Napierian optical absorbance image was calculated based on optical intensity images acquired from 3 articular cartilage sections per sample (with microscope glass) and a reference images (microscope glass only). Depth-wise absorbance was determined at depths 0-100, 100-200, 200-300 μm etc. Absorbance is linearly associated with concentration of light-absorbing medium and is, therefore, used as an indicator for MB content in the tissue.

16 **TABLE A1.** Primer pairs used for droplet digital polymerase chain reactions.

Gene	Protein name	Primer Sequence
<i>ACAN</i>	Aggrecan	Forward: 5' - CGA TAC CCC ATC TCC AAG GC - 3'
		Reverse 3' - AGT GAT ATT TCG GGG CAG GG - 5'
<i>COL2A1</i>	Collagen, type II, alpha 1	Forward: 5' - ACT GCC AGT GGG CAA CCA - 3'
		Reverse 3' - GTC ACC ACG ATC ACC TCT GG - 5'
<i>MMP1</i>	Matrix metalloproteinase 1 (MMP-1)	Forward: 5' - AAA TCC CAC TCA GCC AGT CG - 3'
		Reverse 3' - CCC TGT CGG CAA CCT CAT AA - 5'
<i>MMP3</i>	Matrix metalloproteinase 3 (MMP-1)	Forward: 5' - CAC TCA ACC GAA CGT GAA GC - 3'
		Reverse 3' - GCT GAC TGC ATC GAA GGA CA - 5'
<i>MMP9</i>	Matrix metalloproteinase 9 (MMP-9)	Forward: 5' - GAG ATG CCC ACT TCG ACG AT - 3'
		Reverse 3' - GAG CGA CCC TCA AAG GTG AA - 5'
<i>MMP13</i>	Matrix metalloproteinase 13 (MMP-13)	Forward: 5' - GTT GCT GCC CAT GAG TTT GG - 3'
		Reverse 3' - TGT CTG GCG TTT TGG GAT GT - 5'
<i>TGFB</i>	Transforming Growth Factor Beta	Forward: 5' - AAT AGA GGG CTT TCG CCT CAG - 3'
		Reverse 3' - AGC AGT AGT TGG TGT CCA GG - 5'
<i>TIMP3</i>	Tissue Inhibitor Of Metalloproteinases 3	Forward: 5' - GGC CGA GTC TAT GAT GGC AA - 3'
		Reverse 3' - GTT TGG ACT GGT AGC CAG GG - 5'
<i>TNF</i>	Tumor necrosis factor	Forward: 5' - GAA GAG CAG TCC CCA GGT G - 3'
		Reverse 3' - GGG CTA CCG GCT TGT TAC TT - 5'

17

18