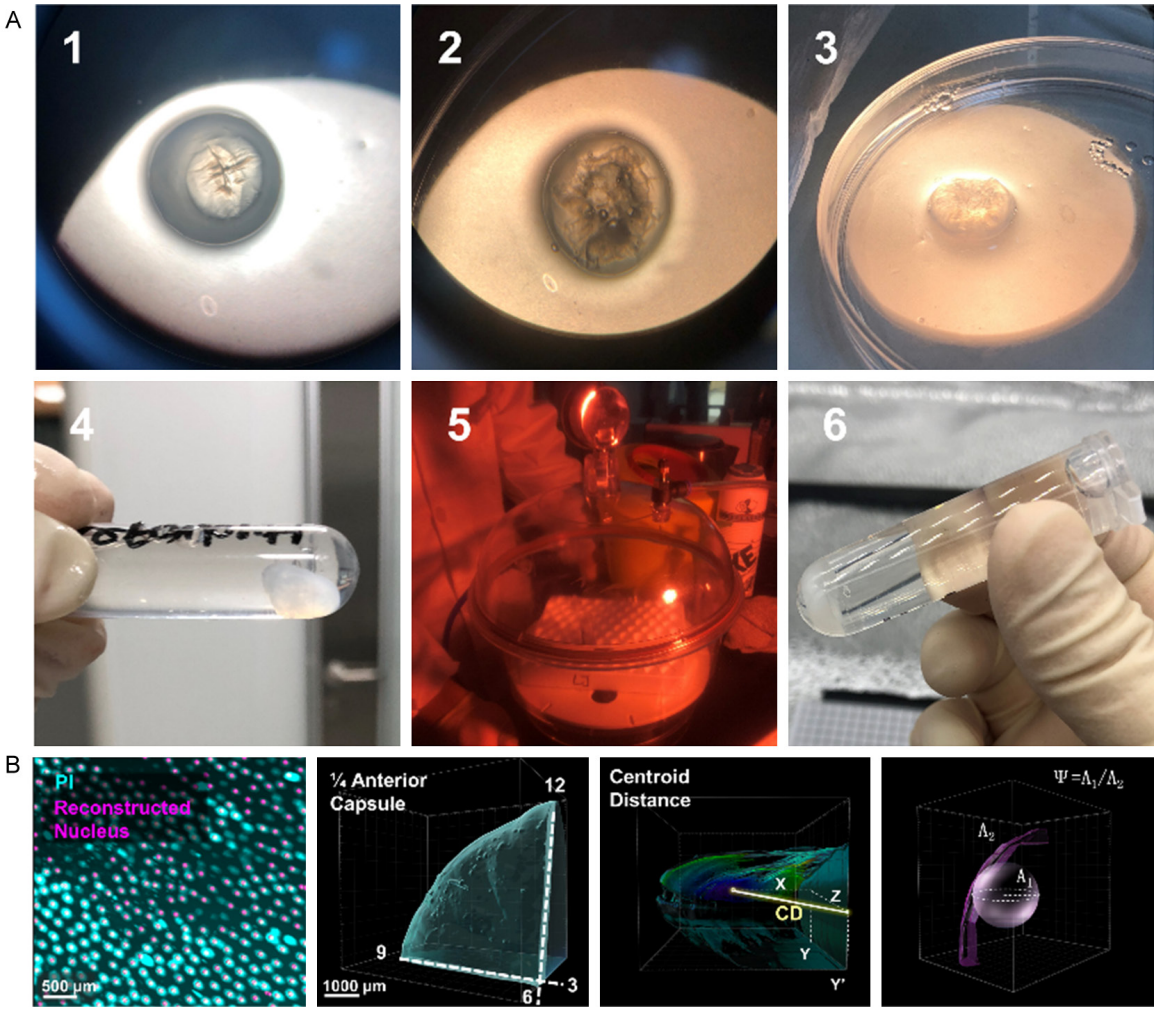
Supplementary Table 1. Reagents, antibodies, equipment, and software

Name	Cat. number	Manufacturer
Reagent		
4% paraformaldehyde (PFA)	CR10010	Crystal-bio, China
PBS	C10010500BT	Gibco, USA
Triton X-100	V900502	Sigma-Aldrich, USA
Sodium azide	S2002	Sigma-Aldrich, USA
e-CLARITY		
Hydrogel solution		
Acrylamide	v900845	Vetec
Bisacrylamide	0172	Amresco
Photoinitiator VA044	va-044/225-02111	Wako
8% SDS solution		
Sodium dodecyl sulfate	30166428	Sinopharm Chemical Reagent
Boric acid	10004818	Sinopharm Chemical Reagent
Sodium hydroxide	10019718	Sinopharm Chemical Reagent
easyIndex	#EI-Z1011	LifeCanvas
CUBIC		
CUBIC clearing solution		
Urea	U5378	Sigma-Aldrich, USA
Tetraethylenediamine	101129	Crystal-bio, China
CUBIC scale solution		
Sucrose	CR101108	Crystal-bio, China
Triethanolamine	101109	Crystal-bio, China
Mineral oil	m8410	Sigma-Aldrich, USA
Silicone oil	TSF-437	Shanghai Puxian, China
iDISCO		
Methanol	34860	Sigma-Aldrich
DCM	270997	Sigma-Aldrich
H_2O_2	D216763	Sigma-Aldrich
Donkey serum	D9663	Sigma-Aldrich
PTwH		
Glycine	G8898	Sigma-Aldrich
DMSO	D8418	Sigma-Aldrich
Tween-20	P9416	Sigma-Aldrich
Heparin	375095	Milipore
Dibenzyl Ether	33630	Sigma-Aldrich
Antibodies		
DAPI	D9542	Sigma-Aldrich
PI	P1304MP	Thermo Fisher Scientific
TrKA	MA5-15509	Invitrogen
ZO-1	33-9100	Invitrogen
Equipment		
Constant temperature incubator	ZHLY-1803	Shanghai Zhichu Instrument, China
Light sheet microscope	LS-18	Nuohai Life Science Co., Ltd., China
Workstation	W-2245	Intel
Software		
Imaris	Imaris 9.7	Bitplane, Switzerland
MATLAB	R2016a	MathWorks, USA
GraphPad Prism	version 8.0.2	GraphPad

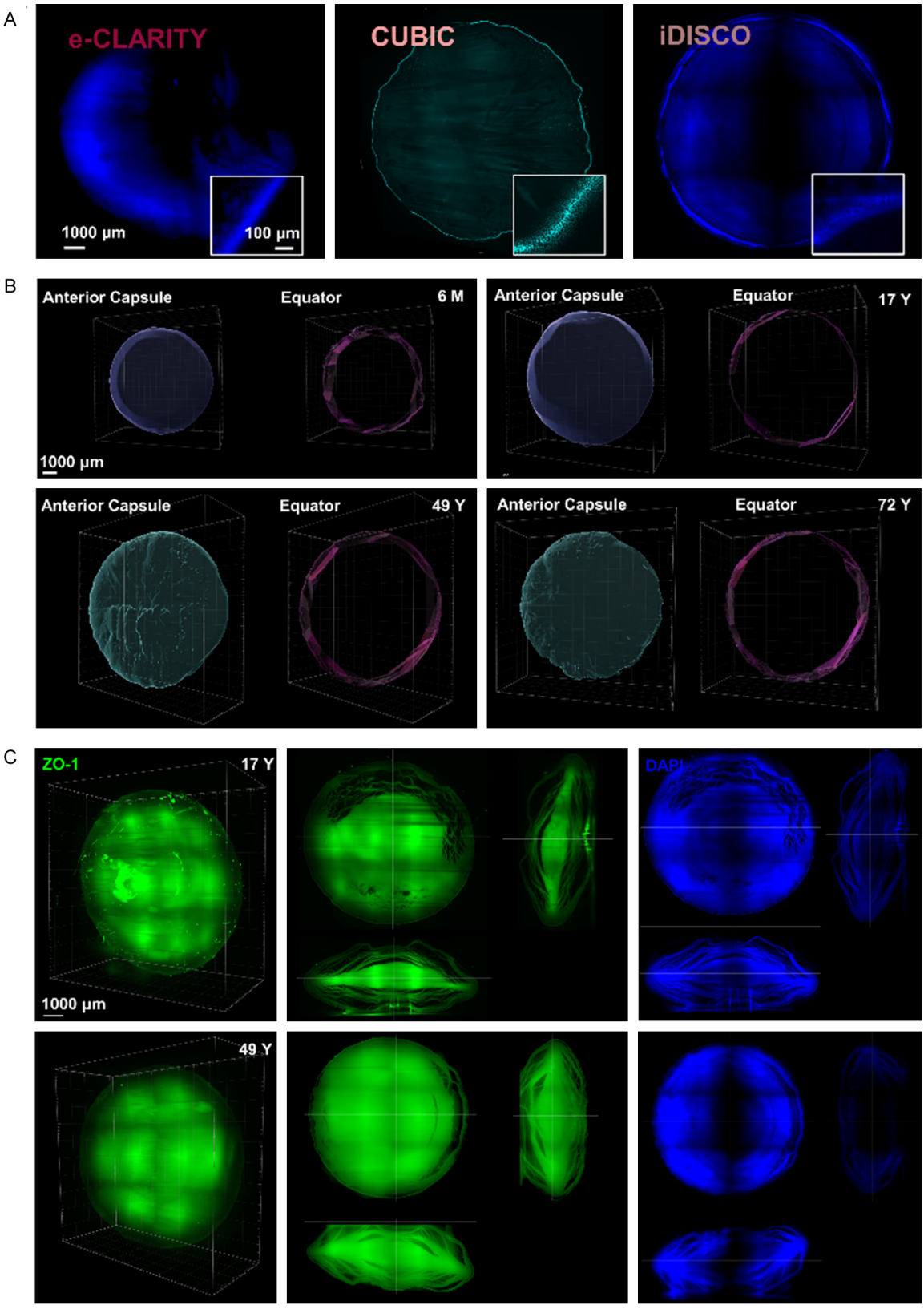
Supplementary Table 2. Tissue clearing and immunostaining methods for each individual lens

Sample	Tissue clearing method	Immunostaining methods
6 M	iDISCO	DAPI, TrkA
17 Y	iDISCO	DAPI, ZO-1
49 Y #1	CUBIC	PI
49 Y #2	iDISCO	DAPI, ZO-1
72 Y #1	CUBIC	PI
72 Y #2	e-CLARITY	DAPI



Supplementary Figure 1. Pretreatment with enucleation and subsequent e-CLARITY 3D-reconstruction. A. Pretreatment with enucleation in the e-CLARITY process. 1 - the isolated lens after removing the central 6 mm of the posterior capsule; 2 - the hollowed-out lens; 3 - the lens filled with hydrogel; 4 - sealing the lens in the Eppendorf tube with hydrogel; 5 - degassing with a vacuum desiccator; and 6 - solidified hydrogel containing the lens. B. Cellular 3D parameters are shown as a schematic diagram of definitions comparing the different ages and different lens parts, including the number of nuclei, the area, the centroid distance, and the sphericity.

3D mapping of human lenses across different ages



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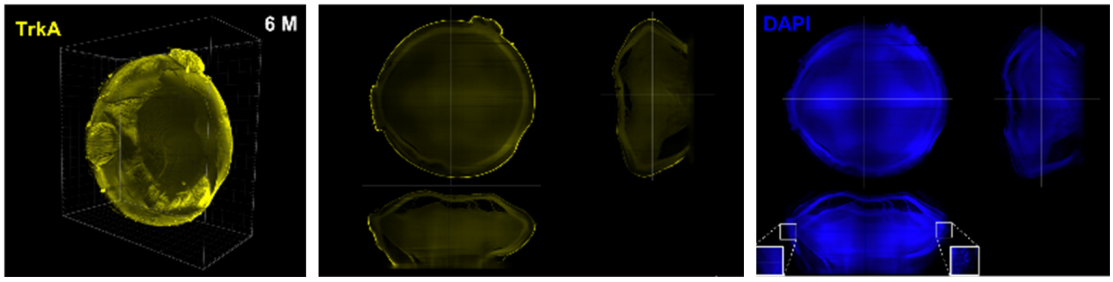

Supplementary Figure 2. Representative 3D views of the molecular and cellular maps of the human lens. A. Randomly chosen planes magnified to show the comparison of nuclear immunostaining efficacy (dark blue for DAPI and light blue for PI) using the different clearing methods. B. Reconstructed surface of the anterior capsule and equator of the 6 M, 17 Y, 49 Y, and 72 Y lenses. C. 3D view of ZO-1 (left) together with three views from the front, side, and top of ZO-1 (middle) and DAPI (right) in addition to the top panel for the 17 Y lens and the bottom panel for the 49 Y lens. D. 3D view of TrkA (left) together with three views from the front, side, and top for TrkA (middle), and DAPI (right) in the 6 M lens.