1   **Title: Spectral Domain Optical Coherence Tomography: An *in vivo* imaging protocol for assessing**

2   **retinal morphology in adult zebrafish**

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11   Conflicts of interest: No author has any conflict of interest.

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26

27 Abstract

28 The present study outlines a protocol for examining retinal structure in zebrafish, a popular model

29 organism for ocular studies, using Spectral Domain Optical Coherence Tomography (SD-OCT). We

30 demonstrate how this live imaging modality can be used to obtain high quality images of several

31 retinal features, including the optic nerve, retinal vasculature and the cone photoreceptor mosaic.

32 Retinal histology sections were obtained from imaged fish for comparison with SD-OCT cross-

33 sectional B-scans. Voronoi domain analysis was used to assess cone photoreceptor packing

34 regularity at 3, 6 and 12 months. SD-OCT is an effective *in vivo* technique for studying the adult

35 zebrafish retina and can be applied to disease models for longitudinal serial monitoring.

36

37 Introduction

38 Zebrafish are gaining increasing prominence as models for the study of vertebrate retinal

39 development and inherited retinal dystrophies [1-4]. The zebrafish retina has a highly organized,

40 multilayered neuronal structure that is conserved across vertebrate species. Like humans, zebrafish

41 are a diurnal species with cone-dominant vision, making them an attractive alternative to other

42 commonly used animal models. Furthermore, zebrafish have a functional visual system by five days

43 post-fertilisation, enabling rapid phenotyping and experimentation. Their external development and

44 optical transparency make them accessible to manipulation at the embryonic stages.

45

46 Among the numerous advantages of studying zebrafish is their genetic manipulability, which has

47 been pivotal in establishing the zebrafish as a biomedical model [5]. The zebrafish genome is well

48 curated and it has been found that approximately 70% of human protein coding genes have at least

49 one zebrafish orthologue [6]. Previously, forward genetic screens using chemical or insertional

50 mutagenesis have provided a host of zebrafish mutant ocular phenotypes for the identification of

51 gene function *in vivo* [7-9]. Many mutations in the zebrafish genome have been associated with

52 phenotypes of visual dysfunction that have parallels with human disease, including models of

53   retinitis pigmentosa and choroideremia [1-4]. More recently, the development of modern genetic

54   techniques, such as TALENs (transcription activator-like effector nucleases) [10, 11] and CRISPR

55   (clustered regularly interspaced short palindromic repeats) [12], have made precise and efficient gene

56   editing possible. The availability of relatively inexpensive and straightforward techniques will

57   facilitate the production of numerous zebrafish mutant lines, modelling a range of retinal

58   developmental and degenerative diseases. As well as providing insight into the underlying

59   pathological details of various disorders, such mutants will serve as preclinical models for assessing

60   the efficacy and safety of potential treatment strategies.

61

62   Currently, the established gold standard for studying retinal structure in zebrafish is *ex vivo*

63   histological assessment. As this technique requires sacrifice of the animal being examined,

64   longitudinal analysis within the same individual is not possible and large numbers of fish may be

65   required to assess multiple different timepoints. Further limitations of traditional histological

66   analysis include the risk of tissue damage and the time-consuming laboratory procedures involved.

67   Alternatively, the use of live imaging modalities, such as Optical Coherence Tomography (OCT) and

68   confocal scanning laser ophthalmoscopy (cSLO), offer both economic and ethical advantages by

69   enabling equivalent images to be obtained in a rapid *in vivo* context.

70

71   OCT is a non-contact, non-invasive imaging technology that can construct detailed cross-sectional

72   and three-dimensional images of the eye [13, 14]. It is the optical analogue of ultrasound. OCT imaging is

73   based on interferometry, where light is sent through a sample arm and a reference arm, and

74   backscattered light from the sample is combined with that from the reference arm to generate an

75   interference signal. Most current generation systems use the spectral properties of wide bandwidth

76   (50-100 nm) light sources in the near infrared range (NIR) to depth resolve the optical properties of a

77   sample. Due to the method of acquisition, these systems are referred to as spectral domain OCT (SD-

78   OCT) [15, 16]. Single depth measurements are called an A-scan, with series of depth scans along a single

79   plane called B-scan, which creates an optical section. Series of densely sampled B-scans can be

80   visualized *en face* to generate optical flat-mounts for further analysis.

81

82   The use of OCT is well-established in the clinical setting, where it is used for diagnosis and

83   monitoring of ophthalmic disease [14]. It has also been used to visualize a variety of animal retinas,

84   including rodents [17, 18], birds [19] and *Xenopus* [20]. Previous studies have successfully used SD-OCT to

85   image ocular tissues in larval, juvenile and adult zebrafish *in vivo* [21-26]. Other tissues, including the

86   brain and heart, have also been examined [21, 27]. In adult zebrafish, it has been shown that detailed,

87   cross-sectional images of the laminated retina and optic nerve are possible, and that SD-OCT can

88   effectively detect degeneration and subsequent regeneration of the inner and outer retinal layers,

89   demonstrated using light- and ouabain-mediated damage paradigms [22, 23, 25]. It has also been used

90   for accurate measurement of several eye dimensions in wild-type and myopia disease models [24].

91   Recently, in combination with cSLO, SD-OCT has been used to closely examine specific layers in outer

92   retina, including the photoreceptors [26]. Although the utility of SD-OCT for zebrafish ocular imaging

93   has been demonstrated, there is limited information available on the protocols used.

94

95   The present study details a protocol for obtaining cross-sectional and *en face* images of the retina in

96   wild-type adult zebrafish, using the Bioptigen Envisu R-series Spectral Domain Ophthalmic Imaging

97   System (SDOIS). The eyes of zebrafish of 3, 6 and 12 months in age were imaged to examine age-

98   related differences in retinal layer thickness and the cone photoreceptor mosaic. Retinal histology

99   sections from imaged fish were also obtained for comparison with SD-OCT cross-sectional images.

100

101   Materials and Methods

102

103   *Animal care*

104   Wild-type (AB) zebrafish were maintained at the University College London (UCL) Institute of

105   Ophthalmology Biomedical Research Unit. The fish were raised to 3, 6 and 12 months of age at

106   28.5°C exposed to 200 lux illuminance for 14 hours daily: 10 hours darkness. A total of 18 fish had

107   SD-OCT imaging carried out on their right eyes. Research was carried out in accordance with the

108   principles and guidelines of The Animals (Scientific Procedures) Act 1986, UK, and the ARVO

109   statement for the Use of Animals in Ophthalmic and Vision Research with local institutional review

110   board approval.

111

112   *Imaging and Animal Equipment*

113   SD-OCT images were captured using the Bioptigen Envisu R2200 SDOIS (Bioptigen Inc., Morrisville,

114   NC.), which is commercially available for small animal imaging. The SD-OCT apparatus included a

115   base system (host computer, SD-OCT engine with reference arm and a hand-held SD-OCT probe),

116   imaging mount and animal alignment stage (see Figure 1). The probe was held in the mount in a

117   vertical position directly above the alignment stage, where the zebrafish was placed, and could be

118   moved up and down. The alignment stage was able to move in the X or Z meridians. InVivoVue

119   software (Bioptigen Inc., Morrisville, NC.) was used for creating and saving OCT image files.

120

121   Prior to all SD-OCT imaging, 4 mg/ml tricaine (Western Chemical Inc., Ferndale, WA) stock solution

122   was diluted to 0.2 mg/ml in tank system water to anaesthetise fish. When unresponsive to touch,

123   anaesthetised fish were transferred for imaging using a plastic spoon. For imaging of the optic nerve,

124   a custom rubber holder with projections to maintain position was used to hold the zebrafish in place

125   (Figure 1B). When imaging the photoreceptor mosaic, the zebrafish were laid on a grooved plasticine

126   wedge placed in an immersion tank with attached tubing and syringe to control water level. A

127   weighted strap was used to stabilise the fish. Surgical tape was used for attaching either the rubber

128   holder or immersion tank to the alignment stage.

129

130   *SD-OCT imaging of the optic nerve and retinal layers*

131   For optic nerve imaging, the zebrafish was placed in the rubber holder and positioned at an angle

132   relative to the probe as demonstrated in Figure 1B. For each fish, a new 'patient' and 'exam' file was

133   created on the InVivoVue programme. A 1.4 mm by 1.4 mm perimeter protocol with 1000 A-scans

134   per B-scan with 100 total scans was used for imaging the optic nerve and retinal lamination. The

135   bore of the SD-OCT probe was initially brought into very close proximity to the fish eye and live

136   imaging was commenced. Following this, the position of the probe relative to the animal stage could

137   be finely adjusted until an adequate image could be obtained and the optic nerve was located. This

138   was achieved by moving the mounted probe up or down, or adjusting the position of the platform

139   with the fish holder. During the imaging process, drops of 0.2 mg/ml tricaine were regularly pipetted

140   on the fish gills to maintain moisture, and on the eye to prevent corneal desiccation and maintain

141   image quality.

142

143   *SD-OCT imaging of the photoreceptor mosaic*

144   When imaging the cone photoreceptor mosaic, the anaesthetised zebrafish was placed in the

145   immersion tank containing 0.2 mg/ml tricaine in tank water solution and was perpendicular to the

146   probe (Figure 1A). A rectangular scanning protocol consisting of a 1 x 1 mm perimeter with 400 A-

147   scans per B-scan with 400 total B-scans was employed for volume intensity projection (VIP) images

148   of the fundus mosaic. The syringe attached to the tank was used to adjust the water level. The

149   optimal water height (approximately 1 mm above the cornea) determined the clarity and brightness

150   of individual photoreceptors. When capturing *en face* images, it was necessary to wait for the fish

151   breathing to become less frequent to reduce breathing artefacts. After imaging, fish were revived

152   and returned to their tank system, unless being used for histological analysis.

153

154   *Histological evaluation*

155   After SD-OCT imaging, three fish per timepoint were euthanised for histological evaluation. The right

156   eye, which underwent imaging, was enucleated and fixed with 4% paraformaldehyde overnight at

157   4°C. Following this, the eyes were washed in phosphate-buffered saline (PBS) and serially

158   dehydrated through a graded ethanol series (30%, 50%, 70%, 95% and 100%) in PBS before

159   embedding in JB-4 resin (Polysciences Inc., Warrington, PA) according to manufacturer's instructions.

160   Using a Leica RM 2065 microtome, 10 μm transverse retinal sections were obtained and stained with

161   1% toluidine blue before imaging on a Zeiss LSM510 upright microscope with AxioCam MRc digital

162   camera.

163

164   *Image analysis*

165   Retinal thickness measurements from SD-OCT B-scans were obtained manually using the Diver

166   software (Bioptigen Inc., Morrisville, NC). For histology images, a stage graticule was imaged at the

167   same magnification for scaling and the equivalent measurements were carried out on transverse

168   retinal sections containing the optic nerve using ImageJ (National Institutes of Health, Bethesda,

169   MD).. Measurements of several easily distinguishable sublayers were taken at a distance of 200 μm

170   and 400 μm from the optic nerve (two points per distance) for each fish retina. The mean and

171   standard deviation of each retinal thickness measurement were calculated per timepoint for both

172   SD-OCT and histology data. Paired t-tests and Bland Altman plotting were used to compare the data

173   sets and assess agreement between the two methods of measurement. Statistical analysis was

174   carried out using JMP12 (SAS, Raleigh, NC). For longitudinal assessment of retinal structure, three

175   fish were imaged on two separate occasions over a three week period and thickness measurements

176   were taken at the same point on the retina, located using the vasculature as a landmark.

177

178   In addition to thickness, the organization of the photoreceptor mosaic was assessed. The ordering

179   present in a photoreceptor array can be analysed by several methods including nearest-neighbour

180   method [28], neuron density [28] and Voronoi tessellation [29] to provide a statistical assessment of the

181   regularity in a receptor array. Metrics such as nearest neighbour and neuron density require

182   information regarding image magnification and size of the region of interest. Since optical models of

183 the fish eye do not exist, and a fish eye size varies greatly with age, these metrics were not

184 applicable to our research. Therefore, Voronoi domain analysis was selected to better describe the

185 orderliness with which the receptor array tiles the retina [30, 31]. In this kind of tiling, all points in

186 the plane are partitioned into Voronoi domains which represent all those points in the plane that

187 are closer to a particular cell than to any other cell. The ideal sampling of a mosaic is produced

188 by hexagonally, 6-sided, arranged photoreceptor cells. In this study, photoreceptor cells were

189 manually identified using ImageJ from *en face* SD-OCT scans. Cell coordinates were analysed using

190 custom MATLAB software (MATLAB, MathWorks, Natick, MA) [30, 32]. Percent 6-sided cells and

191 distribution of sidedness were assessed.

192

193 Results

194

195 *Imaging of the optic nerve, retinal lamination and vasculature*

196 Using our SD-OCT equipment set up, it was possible to obtain detailed cross-sectional views of the

197 adult zebrafish retina with clearly delineated layers - ganglion cell layer (GCL), inner plexiform layer

198 (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), photoreceptor

199 layer, retinal pigment epithelium (RPE) - and optic nerve (Figure 2). The optic nerve, which can be

200 used as a retinal landmark to orient the scans, appeared as a smudge-like interruption to the linear

201 arrangement of the retinal layers (Figure 2A). In the *en face* projection, the inner retinal blood

202 vessels could be visualized travelling outwards from the optic nerve region and branching towards

203 the peripheral retina (Figure 2B).

204

205 *SD-OCT versus histology for assessment of retinal structure*

206 By comparing SD-OCT retinal B-scans with retinal histology sections, we have shown that live

207 imaging can provide an accurate representation of the structural organization of the zebrafish retina,

208 with a level of detail akin to that obtained by histological methods (Figure 3). Optimising the level of

209   water over the cornea of the eye being imaged produced greater definition in the retinal sublayers,

210   particularly the outer layers in which the external limiting membrane (ELM) and photoreceptor outer

211   segments (OS) could be distinguished.

212

213   Measurement of individual retinal sublayers (GCL, IPL) or grouped sublayers (INL-ONL, GCL-ELM) was

214   carried out on both SD-OCT and histology images of wild-type retinas at 3, 6 or 12 months of age

215   (Table 1). Mean thickness values obtained from the two methods were similar for each timepoint.

216   The mean GCL-ELM thicknesses taken from SD-OCT and histology images were 129 μm (±8.0) and

217   129 μm (±8.8) respectively. Between the 3, 6 and 12 month time points, both data sets showed that

218   retinal layer thicknesses varied minimally, and GCL-ELM measurements showed a modest overall

219   increase from 3 to 12 months (4 μm and 12 μm on SD-OCT and histology images respectively). Using

220   paired t-tests to compare all SD-OCT and histology data for each retinal measurement, there was no

221   significant difference between most layers (GCL, p=0.2; INL-ONL, p=0.074; GCL-ELM, p=0.28).

222   Interestingly, the IPL thickness was significantly different between the two forms of assessment

223   (p=0.0318).

224

225   Bland Altman analysis was used to calculate the mean differences between SD-OCT and histology

226   measurements and to estimate the confidence interval (CI) within which 95% of the differences lie.

227   For GCL, IPL, INL-ONL and GCL-ELM thickness measurements, the mean differences between SD-OCT

228   and histology were 2 μm (95% CI, -18 – 22 μm), -4 μm (95% CI, -20 – 13 μm), 3 μm (95% CI, -18 – 20

229   μm) and 2 μm (95% CI, -30 – 33 μm) respectively. All time point data were analysed together as

230   separate Bland Altman plots did not reveal age-related biases between the measurements.

231

232   *Longitudinal assessment of retinal thickness*

233   To examine the ability of SD-OCT to obtain reproducible longitudinal data within the same animal,

234   repeat imaging and retinal thickness measurements at the same approximate point, using

235   vasculature as a landmark, were carried out on three wild-type zebrafish over the course of three

236   weeks (Figure 4). The point of measurement was located each time using inner retinal vessel

237   branching patterns on the *en face* projection as a reference. Using this method, we found that

238   thickness values obtained from separate imaging sessions were relatively consistent within

239   individual fish. The mean measurements (± standard deviation) for the GCL, IPL and GCL-IPL were

240   respectively as follows: 37 µm (±2.6), 26 µm (±2.3) and 107 µm (±13.3) at week 0, and 38 µm (±2.6),

241   27 µm (±3.8) and 108 µm (±14.5) at week 3.

242

243   *Examination of the cone photoreceptor mosaic*

244   The highly ordered spatial organization of photoreceptors is likely essential to maximise vision. The

245   adult zebrafish retina has four cone photoreceptor subtypes, differing in their spectral sensitivity,

246   which are arranged into a precise, reiterated pattern (mosaic) with tiering [33, 34]. The well stereotyped

247   mosaic organization consists of alternating rows of red/green- sensitive double cones and ultraviolet

248   (UV)- and blue- sensitive single cones (Figure 5). Using SD-OCT imaging, we demonstrated that by

249   analysing specific regions within the photoreceptor layer on the B-scan retinal cross-section (Figure

250   5A & 5C), it was possible to visualize the innermost and outermost cone tiers, the presumptive UV

251   and red/green submosaics, on the corresponding *en face* VIP views (Figure 5B, D). By merging these

252   cone layers, a detailed image of the precisely organized adult zebrafish cone mosaic was constructed

253   (Figure 5F).

254

255   Voronoi domain analysis was used to assess the regularity of the wild-type UV cone mosaic at 3, 6

256   and 12 months of age (Figure 6). Only regions of the peripheral retina containing the adult mosaic

257   growth were analysed and the disorganized larval remnant was excluded. Voronoi diagrams, in

258   which a Voronoi polygon is associated with each cone photoreceptor and color-coded according to

259   the number of sides it possesses, were derived from the cone mosaic images (Figure 6B). The

260   zebrafish retinas at each timepoint were dominated by regions of green-coded 6-sided polygons,

261 indicating a regular triangular lattice. The other colors marked points of disruption in the

262 hexagonally packed mosaics. The presence of more numerous, smaller domains highlighted a clear

263 increase in cone cell number in the 12 month mosaic compared to that of 3 and 6 months.

264 Assessment of the distribution of sidedness in the Voronoi domains for each timepoint (n=3)

265 demonstrates that there was minimal variation of the cone mosaic arrangement, maintaining its

266 regularity with age (Figure 6C). Overall, the number of sides was found to range between 4 and 9. At

267 3, 6 and 12 months, the mean percentage of cones with 6 neighbours were 75.4%, 69.7% and 69.7%

268 respectively, indicative of mosaics with mostly regular hexagonal cone packing. Greater

269 disorganization in the pattern is associated with lower percentages of 6-sided domains. The

270 reduction in regularity with age is predicted by normal loss of photoreceptors with age.

271

272 Discussion

273 SD-OCT is an important imaging modality used extensively in the clinical practice of ophthalmology.

274 It has also become increasingly popular in the laboratory setting, as a non-invasive, cost-effective

275 alternative to *ex vivo* assessment of animal retinal structure. By reducing the number of animals

276 necessary for experimentation, the use of such live imaging is in keeping with the guiding principles

277 for ethical use of animals in research, known as the 'three Rs' (replacement, refinement and

278 reduction). Several studies have already applied the technique to zebrafish, and have shown its

279 ability to form accurate representations of their ocular tissues [21-26]. Here, we have provided a

280 practical and reproducible protocol for capturing high quality SD-OCT images of various retinal

281 features, including the optic nerve, retinal vasculature and photoreceptor mosaic, in wild-type adult

282 zebrafish.

283

284 Using a commercially available SD-OCT device, we have demonstrated how *in vivo* imaging can be

285 used to qualitatively and quantitatively assess the cross-sectional views of the zebrafish retinal

286 lamination, providing a level of detail comparable to that of plastic resin-embedded histology

287   sections (Figure 3 and Table 1). Retinal layer thickness values obtained from histology followed the

288   same overall trend of mild growth with age found by SD-OCT measurements between 3 and 12

289   months. Previously, it was shown that the retinal radius and other eye measurements continue to

290   increase throughout the zebrafish lifetime [24]. Comparative analysis between SD-OCT and histology

291   data showed that the two techniques produced similar results for GCL, INL-ONL and GCL-ELM

292   thicknesses and mean differences were relatively small, ranging from -4 to 3 µm. Prior studies in

293   rodents and zebrafish have found good correlations between *in vivo* and *ex vivo* retinal

294   measurements [18, 22, 35], although some inconsistency between the two methods from normal,

295   damaged and regenerative zebrafish retinas were reported [22]. Many factors may contribute to the

296   discrepancy, including artefacts arising from histology procedures, such as tissue swelling, shrinking

297   and tearing [35-37]. The retinal sublayers appear to be differentially affected by these processes, with

298   IPL thickness being significantly different on SD-OCT and histology images, which may be related to

299   the fact that it is a relatively large synaptic layer while the other measured layers (GCL, INL-ONL)

300   have greater cell body content. Despite the disparity in IPL thicknesses, GCL-ELM measurements

301   were still very similar between the *in vivo* and *ex vivo* data. It is likely that SD-OCT imaging provides a

302   more accurate depiction of the live zebrafish retina, due to its ability to acquire two- and three-

303   dimensional information in a live, unprocessed state. However, although SD-OCT provides a rapid

304   and repeatable method for screening ocular phenotypes, the equipment is costly and it cannot

305   replace histological techniques for imaging the morphology of the various retinal cells and how they

306   interact, particularly the outer segments and RPE. In our study, we have used *ex vivo* data for

307   comparison with SD-OCT retinal thickness measurements using the DIVER software, which corrects

308   for dispersion and optical effects of the system. But for most accuracy it would be necessary to

309   measure the axial length of the eye to correct for optical magnification *in vivo*. Such calibration is

310   not required for assessing photoreceptor organization.

311

312   The adult zebrafish cone photoreceptor mosaic has a highly ordered spatial organization, consisting

313   of four cone spectral subtypes packed into a reiterative lattice arrangement [33]. Examination of this

314   retinal feature both *ex vivo* and *in vivo* has typically involved the use of fluorescent labelling and

315   transgenic lines [33, 38, 39]. In our study, we have used SD-OCT to capture high definition *en face*

316   projections of the cone mosaic and demonstrated its ability to distinguish specific cone sublayers

317   (Figure 5). Visualization of the blue cone and rod photoreceptors was difficult using our equipment

318   and higher resolution systems will likely improve the ability to distinguish these cells [26]. Using

319   Voronoi analysis, it was possible to quantitatively assess the regularity of the wild-type UV cone

320   mosaic at different ages from SD-OCT images (Figure 6). The percentage of six-sided Voronoi

321   domains was around 70% at all three timepoints examined, indicative of regular hexagonal cone

322   packing observed in healthy retinas [30]. Overall, our results suggest that using SD-OCT data to perform

323   mosaic analysis could be a feasible and robust method for assessing longitudinal changes in

324   photoreceptor organization in zebrafish disease models compared to wild-type.

325

326   Zebrafish continue to grow in popularity as models of human degenerative retinal disorders.

327   Additionally, they offer a relatively inexpensive alternative to high maintenance mammalian models

328   for assessing the safety and efficacy of new drug compounds and treatments. The burgeoning use of

329   zebrafish will place increasing demand on developing rapid and cost-effective *in vivo* means of

330   studying the zebrafish retina. SD-OCT is an excellent tool for non-invasive, longitudinal examination

331   of various aspects of the zebrafish ocular morphology, and the use of this technique is likely to

332   greatly develop over the coming years.

333

341   <u>References</u>

342

343   1.   Gestri G, Link BA and Neuhauss SC. The visual system of zebrafish and its use to model

344   human ocular diseases. Dev Neurobiol 2012;72:302-27.

345   2.   Chhetri J, Jacobson G and Gueven N. Zebrafish--on the move towards ophthalmological

346   research. Eye (Lond) 2014;28:367-80.

347   3.   Link BA and Collery RF. Zebrafish Models of Retinal Disease. Annual Review of Vision Science

348   2015;1:125-153.

349   4.   Richardson R, Tracey-White D, Webster A and Moosajee M. The zebrafish eye-a paradigm

350   for investigating human ocular genetics. Eye (Lond) 2016. [Epub ahead of print]

351   5.   Varshney GK, Sood R and Burgess SM. Understanding and Editing the Zebrafish Genome. Adv

352   Genet 2015;92:1-52.

353   6.   Howe K, Clark MD, Torroja CF, Torrance J, Berthelot C, Muffato M, et al. The zebrafish

354   reference genome sequence and its relationship to the human genome. Nature

355   2013;496:498-503.

356   7.   Haffter P, Granato M, Brand M, Mullins MC, Hammerschmidt M, Kane DA, et al. The

357   identification of genes with unique and essential functions in the development of the

358   zebrafish, Danio rerio. Development 1996;123:1-36.

359   8.   Malicki J, Neuhauss SC, Schier AF, Solnica-Krezel L, Stemple DL, Stainier DY, et al. Mutations

360   affecting development of the zebrafish retina. Development 1996;123:263-73.

361   9.   Gross JM, Perkins BD, Amsterdam A, Egana A, Darland T, Matsui JI, et al. Identification of

362   zebrafish insertional mutants with defects in visual system development and function.

363   Genetics 2005;170:245-61.

364　10.　Huang P, Xiao A, Zhou M, Zhu Z, Lin S, and Zhang B. Heritable gene targeting in zebrafish

365　　　using customized TALENs. Nat Biotechnol 2011;29:699-700.

366　11.　Sander JD, Cade L, Khayter C, Reyon D, Peterson RT, Joung JK, et al. Targeted gene disruption

367　　　in somatic zebrafish cells using engineered TALENs. Nat Biotechnol 2011;29:697-8.

368　12.　Hwang WY, Fu Y, Reyon D, Maeder ML, Tsai SQ, Sander JD, et al. Efficient genome editing in

369　　　zebrafish using a CRISPR-Cas system. Nat Biotechnol 2013;31:227-9.

370　13.　Huang D, Swanson EA, Lin CP, Schuman JS, Stinson WG, Chang W, et al. Optical coherence

371　　　tomography. Science 1991;254:1178-81.

372　14.　Adhi M and Duker JS. Optical coherence tomography--current and future applications. Curr

373　　　Opin Ophthalmol 2013;24:213-21.

374　15.　Wojtkowski M, Leitgeb R, Kowalczyk A, Bajraszewski T and Fercher AF. In vivo human retinal

375　　　imaging by Fourier domain optical coherence tomography. Journal of biomedical optics

376　　　2002;7:457-63.

377　16.　Sull AC, Vuong LN, Price LL, Srinivasan VJ, Gorczynska I, Fujimoto JG, et al. Comparison of

378　　　spectral/Fourier domain optical coherence tomography instruments for assessment of

379　　　normal macular thickness. Retina 2010;30:235-45.

380　17.　Srinivasan VJ, Ko TH, Wojtkowski M, Carvalho M, Clermont A, Bursell SE, et al. Noninvasive

381　　　volumetric imaging and morphometry of the rodent retina with high-speed, ultrahigh-

382　　　resolution optical coherence tomography. Invest Ophthalmol Vis Sci 2006;47:5522-8.

383　18.　Huber G, Beck SC, Grimm C, Sahaboglu-Tekgoz A, Paquet-Durand F, Wenzel A, et al. Spectral

384　　　domain optical coherence tomography in mouse models of retinal degeneration. Invest

385　　　Ophthalmol Vis Sci 2009;50:5888-95.

386　19.　Ruggeri M, Major JC, Jr., McKeown C, Knighton RW, Puliafito CA, and Jiao S. Retinal structure

387　　　of birds of prey revealed by ultra-high resolution spectral-domain optical coherence

388　　　tomography. Invest Ophthalmol Vis Sci 2010;51:5789-95.

389   20.   Lee DC, Xu J, Sarunic MV and Moritz OL. Fourier domain optical coherence tomography as a

390   noninvasive means for in vivo detection of retinal degeneration in Xenopus laevis tadpoles.

391   Invest Ophthalmol Vis Sci 2010;51:1066-70.

392   21.   Kagemann L, Ishikawa H, Zou J, Charukamnoetkanok P, Wollstein G, Townsend KA, *et al.*

393   Repeated, noninvasive, high resolution spectral domain optical coherence tomography

394   imaging of zebrafish embryos. Mol Vis 2008;14:2157-70.

395   22.   Bailey TJ, Davis DH, Vance JE and Hyde DR. Spectral-domain optical coherence tomography

396   as a noninvasive method to assess damaged and regenerating adult zebrafish retinas. Invest

397   Ophthalmol Vis Sci 2012;53:3126-38.

398   23.   Weber A, Hochmann S, Cimalla P, Gartner M, Kuscha V, Hans S, *et al.* Characterization of

399   light lesion paradigms and optical coherence tomography as tools to study adult retina

400   regeneration in zebrafish. PLoS One 2013;8:e80483.

401   24.   Collery RF, Veth KN, Dubis AM, Carroll J and Link BA. Rapid, accurate, and non-invasive

402   measurement of zebrafish axial length and other eye dimensions using SD-OCT allows

403   longitudinal analysis of myopia and emmetropization. PLoS One 2014;9:e110699.

404   25.   DiCicco RM, Bell BA, Kaul C, Hollyfield JG, Anand-Apte B, Perkins BD, *et al.* Retinal

405   regeneration following OCT-guided laser injury in zebrafish. Invest Ophthalmol Vis Sci

406   2014;55:6281-8.

407   26.   Bell BA, Yuan A, Dicicco RM, Fogerty J, Lessieur EM, and Perkins BD. The adult zebrafish

408   retina: In vivo optical sectioning with Confocal Scanning Laser Ophthalmoscopy and Spectral-

409   Domain Optical Coherence Tomography. Exp Eye Res 2016;153:65-78.

410   27.   Zhang J, Ge W and Yuan Z. In vivo three-dimensional characterization of the adult zebrafish

411   brain using a 1325 nm spectral-domain optical coherence tomography system with the 27

412   frame/s video rate. Biomedical optics express 2015;6:3932-40.

413   28.   Wassle H and Riemann HJ. The mosaic of nerve cells in the mammalian retina. Proc R Soc

414   Lond B Biol Sci 1978;200:441-61.

415   29.   Shapiro MB, Schein SJ and de Monasterio FM. Regularity and Structure of the Spatial Pattern

416   of Blue Cones of Macaque Retina. Journal of the American Statistical Association

417   1985;80:803-812.

418   30.   Baraas RC, Carroll J, Gunther KL, Chung M, Williams DR, Foster DH, et al. Adaptive optics

419   retinal imaging reveals S-cone dystrophy in tritan color-vision deficiency. J Opt Soc Am A Opt

420   Image Sci Vis 2007;24:1438-47.

421   31.   Kram YA, Mantey S and Corbo JC. Avian cone photoreceptors tile the retina as five

422   independent, self-organizing mosaics. PLoS One 2010;5:e8992.

423   32.   Cooper RF, Sulai YN, Dubis AM, Chui TY, Rosen RB, Michaelides M, et al. Effects of Intraframe

424   Distortion on Measures of Cone Mosaic Geometry from Adaptive Optics Scanning Light

425   Ophthalmoscopy. Translational vision science & technology 2016;5:10.

426   33.   Allison WT, Barthel LK, Skebo KM, Takechi M, Kawamura S, and Raymond PA. Ontogeny of

427   cone photoreceptor mosaics in zebrafish. J Comp Neurol 2010;518:4182-95.

428   34.   Branchek T and Bremiller R. The development of photoreceptors in the zebrafish,

429   Brachydanio rerio. I. Structure. J Comp Neurol 1984;224:107-15.

430   35.   Ferguson LR, Grover S, Dominguez JM, 2nd, Balaiya S and Chalam KV. Retinal thickness

431   measurement obtained with spectral domain optical coherence tomography assisted optical

432   biopsy accurately correlates with ex vivo histology. PLoS One 2014;9:e111203.

433   36.   Gloesmann M, Hermann B, Schubert C, Sattmann H, Ahnelt PK, and Drexler W. Histologic

434   correlation of pig retina radial stratification with ultrahigh-resolution optical coherence

435   tomography. Invest Ophthalmol Vis Sci 2003;44:1696-703.

436   37.   Frenkel S, Morgan JE and Blumenthal EZ. Histological measurement of retinal nerve fibre

437   layer thickness. Eye (Lond) 2005;19:491-8.

438   38.   Salbreux G, Barthel LK, Raymond PA and Lubensky DK. Coupling mechanical deformations

439   and planar cell polarity to create regular patterns in the zebrafish retina. PLoS Comput Biol

440   2012;8:e1002618.

441   39.   Duval MG, Chung H, Lehmann OJ and Allison WT. Longitudinal fluorescent observation of

442   retinal degeneration and regeneration in zebrafish using fundus lens imaging. Mol Vis

443   2013;19:1082-95.
444

445