Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

eAppendix 1. ROP descriptors: benefits and limitations for drug development

A prerequisite for any future ROP trial is to ensure that the disease descriptors are robust. The International Classification of Retinopathy of Prematurity (ICROP) has played a pivotal role in describing ROP in practice and research. It is pertinent to note that several features of ICROP have been pragmatically, rather than scientifically, derived. Furthermore, the descriptions of certain features are ambiguous, open to interpretation [16,20] and may not meet future needs [21]. When developing descriptors, certain features are described pragmatically and the concept of a sign being absolutely correct is possible but not assured. However, variability can be minimized by consensus agreement on common data elements. To achieve this, the description of those features of ICROP which are either ambiguous or insufficiently sensitive to meet the requirements for future trials need to be modified.

Currently there can be uncertainties with respect to an ROP diagnosis and the specific indications for treatment. It is important that these are eliminated to ensure the robustness of clinical data in future therapeutic trials. Table 3 summarizes suggestions for more robust descriptors for use in future clinical trials.

Table 3

ROP - Acute Phase Descriptors		**Comment**
Zone		
Zone I	Assessed by its temporal border only a) by indirect ophthalmoscopy - assessed by 28 diopter lens –nasal edge of disc at	

	one border of field of view with temporal edge at other border b) by digital imaging – twice optic disc-foveal distance	
Zone II - posterior	Assessed by its temporal border only Up to 3 X the optic nerve-fovea distance	
Zone II - anterior	Assessed by its nasal border only Extent of vascularisation of nasal-most 2 clock hours to within 1 disc diameter of the ora serrata	
Zone II but notch in Z I	Record as "Zone II w/ notch.	Zone II ROP with an incursion <1 clock hour into zone I, is designated as Zone II
Zone III	Residual crescent to zone II	
Stage		
Stages 1, 2	Unchanged from ICROP definition	
Stage 3	Unchanged from ICROP definition But better if subdivided by severity	Need for reproducible quantification of severity
APROP	If no signs of stage 3 record as APROP	
APROP & Stage 3	APROP and stage 3 in one eye – APROP/ stage3	APROP & stage 3 are not necessarily distinct entities & can coexist

Stages 4 & 5	Unchanged from ICROP definition	
Extent		
Each stage recorded by quadrant of the retinal circumference		
Plus spectrum		The need for reproducible quantification of plus is acknowledged
No plus nor preplus	Record as normal	
Preplus	Vessel dilatation & tortuosity but insufficient for plus	
Plus	(sufficient dilatation and tortuosity ≥2 quadrants) subdivided by severity	
Post-plus	Remaining tortuosity and dilatation for example after anti-VEGF therapy	After rapid resolution of plus disease, post-plus disease resembles pre-plus and is often very slow to fully recede or stabilize

The descriptions are based on indirect ophthalmoscopy and digital imaging. Fluorescein angiography may increase the sensitivity to stage 3 diagnosis [22–24].

The Plus Spectrum

QUALITATIVE ASSESSMENT - Plus disease comprises venous dilatation and arteriolar tortuosity at the posterior pole, in 2 or more quadrants, that meets or exceeds the degree of abnormality seen in the standard photograph of plus disease. The term pre-plus was added in 2005 to ICROP to describe an intermediate degree of activity, of which approximately 70% progress to plus and require treatment, at a mean of 1.6 weeks [25]. It is important to note that there can also be "post-plus" or remaining tortuosity and dilation after anti-VEGF treatment, which does not carry the same implications for disease progression. This occurs often after resolution of plus disease and resembles pre-plus. It is often very slow to recede or to stabilize.

Except for one rare ROP subtype, plus disease is now the driver for ROP treatment. Thus, it is critical for the Ophthalmologist to recognize the signs of plus. However, this does not always occur as experts cannot agree on what constitutes normal retinal vasculature, preplus and plus disease. There is actually greater agreement on severe disease. There is a recent trend to diagnose preplus and plus at earlier stages of ROP [26]. For convenience (such as anesthesia for other reasons) treatment may be undertaken before the recommended indications are met [27–29]. To introduce greater granularity, the plus spectrum would be best subdivided into: pre-plus, mild, moderate and severe plus (that should be based in the future on consensus-agreed images). Although this is an imperfect approach, an interim measure is needed until quantifying plus disease becomes possible in routine practice.

QUANTIFYING PLUS – There is an urgent need to progress from qualitative assessments to quantitative measurements of plus. Methods applied to ROP include Retinal Image multiScale Analysis (RISA [30–32]), ROPtool [33], Computer-Aided Image Analysis of the Retina (CAIAR [34]), NEAT [35], i-ROP [16], and other methods[36].

The current state on the measurement of retinal vessels and plus:

- Vascular tortuosity can be measured accurately; vessel width is more challenging and dependent on image quality
- Can be used to monitor vessel parameters in progression [37] and following treatment.
- Analysis of narrow field images arterioles and venules together can be obtained by non-physicians and broadly predict the severity of peripheral ROP [38]. But,
- Most measurement methods are based on a clinical diagnosis of plus, rather than on a quantifiable scale.
- Methodology not yet applicable for general use

Summary

In order to meet the requirements of future research, there is a need to improve data collection for the acute phase ROP and agree on common data elements to collect. Indirect ophthalmoscopy and digital imaging will remain the core clinical tools with the future automated analysis of the plus spectrum as an important component of the image analysis. Other invasive methods such as fluorescein angiography and noninvasive methods such as optical coherence tomography show promise in specialized situations.

eAppendix S. Methodology for fundus photography during screening examinations

For the acquisition of interpretable fundus photographs, it is important to perform the following:

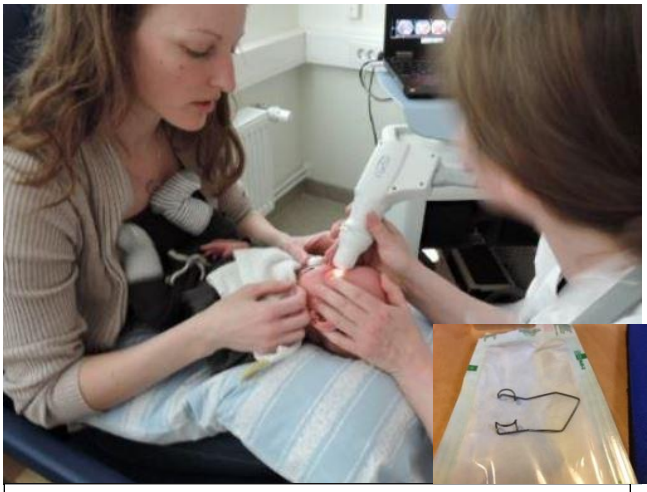


Figure 1; Positioning of infant, parent and child for fundus photos; lid speculum used

- Place the baby in the parent's lap on a large pillow with the head away from the parent.
- Dilate pupils well before the examination. Occlusion of the lacrimal punctae during administration reduces systemic side effects
- The eyes are anaesthetized topically in each eye
- A lid speculum inserted with contact gel is recommended for best examination results.
- Photograph the temporal, superior, inferior and nasal retina then photograph the central fundus. This results in 5 images/eye and normally takes less than 30 seconds in total. For complete coverage of the vascularized retina, 10 overlapping images are required. For more detailed descriptions of how to obtain fundus photographs see references [39–41]
- The infant can be examined in the same way even if the baby is receiving respiratory support.
- If the infant is very weak, concentrate on a quick central image.
- The infant can be given glucose water, formula, or breast milk according to routine clinical practice. It can also be administered to infants receiving respiratory support.
- Interrupt the examination if the heart rate is increasing/decreasing out of normal range or if the infant shows any other signs of significant stress e.g low tone, apnea or decreasing oxygen saturation.
- The parents should receive a preliminary result directly after the examination.

Figure titles

eFigure 1 Appendix B Positioning of infant, parent and child for fundus photos and lid speculum used

eAppendix 3. Overview of OCT for acute assessment and structural outcomes in ROP

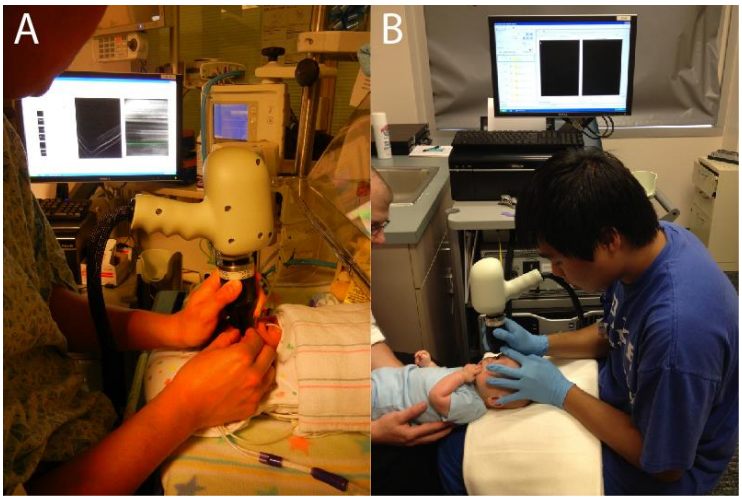
Advanced imaging technology will be useful in addressing our two challenges:

1. Collecting robust data on acute phase ROP which measures the response to novel treatments
2. Setting robust short and long-term structural outcome measures for clinical trials.

Structural assessments of ROP are based on clinical examination or as documented on retinal photographs. In many locations where ROP care is provided, there is limited access to advanced technology such as ultrasound, fluorescein angiography and optical coherence tomography (OCT). Ultrasound is a standard tool for the evaluation of detached retina through unclear media, such as for Stage 4 and 5 ROP in the presence of vitreous hemorrhage. Fluorescein angiography is described in eAppendix D.

Optical coherence tomography uses reflected light to provide cross sectional information on retinal microanatomy. Spectral domain (SD)OCT, because of the high acquisition speed and less than 5 micrometer resolution, is useful in determining structural posterior retinal findings and reproducible measurements of thicknesses, typically of the retinal nerve fiber layer, surrounding retina and macula (equivalent to within zone I), which cannot be appreciated clinically or with digital photography in many retinal diseases. SDOCT assessments are well-established for use in treatment decisions and as endpoints in clinical trials and management of retinal vascular diseases. In ROP, SDOCT imaging has the demonstrated potential to contribute

to acute assessment of ROP activity, to subdivide the disease staging, and for the assessment of structural outcomes. All of these distinctions are important in reporting on the disease effects with the use of a new drug.



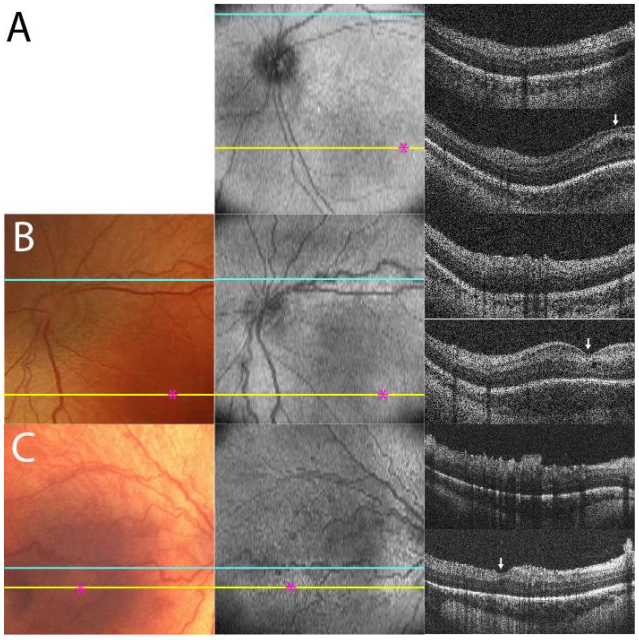
eFigure 2. Photographs of imager setup for portable OCT imaging in the intensive care nursery and outpatient clinic.

Handheld OCT imaging uses near infrared light, does not touch the infant and is conducted comfortably in the isolette in the neonatal intensive care unit (A) and the outpatient pediatric ophthalmology clinic (B). Videos of imaging are in reference.

A recent advancement, OCT angiography (OCT-A) does not require the use of dyes and provides imaging of retinal vascular flow. This permits imaging similar to fluorescein angiography except without visualization of vascular leakage, as shown in studies of the foveal capillaries in children with a history of ROP [42]. OCT angiography is in investigational applications in infants and is likely to have use in future assessment of the vasculature in ROP [43,44].

Currently OCT imaging in preterm infants and the use of these images in the grading of ROP and ROP endpoints are in their infancy in contrast to clinical examination or digital photographic data. Unlike these other modalities, OCT imaging is non-contact and uses infrared light, making it comfortable for infants in the nursery (eFigure 2A) as well as the outpatient setting (eFigure 2B, videos of OCT imaging in Rothman et al [45]). Most OCT data are from

single center studies and findings have not been validated through larger prospective studies or across multiple centers. OCT imaging beyond zone I has been demonstrated in infants, but has not been tested across imagers. Noting these caveats, there are numerous structural findings from OCT that are pertinent to ROP disease structural scale. These are divided into (a) structural findings visible on SDOCT which reflect ROP disease activity (eFigures 3-5), (b) ROP structural outcomes (eFigures 4-5) and (c) features which may reflect the prematurity of the infant retina and/or impact of ROP or therapeutics on the premature retina and retinal development (eFigure 5B).



eFigure 3. Comparison between color photographs and OCT retinal and cross-sectional views of the infant eye with ROP.

In preterm infant eyes SDOCT imaging captures the retinal vascular pattern on the retinal view (center column) which is comparable to Retcam fundus photographs (left column), and also provides cross-sectional information across the posterior pole. The ophthalmologist described the vessels as no plus (A), pre-plus (B) and plus disease (C). Based on the SDOCT appearance in cross section, the location of the foveal center can be determined and is designated with a white arrow on the SDOCT cross-sectional image and with a pink star on the SDOCT retinal view, and copied onto the fundus photo. Tortuous retinal vessels deform the inner retina in cross section in (B) and (C), and preretinal neovascular tissue extends over the posterior retina in the cross sections in (C).

(a) The following SDOCT retinal features reflect ROP disease structure/activity. New technology points to the future utility this imaging in more peripheral ROP in these infants [43] (eFigure 3)

1) foveal center (to estimate zone I, eFigure 3)

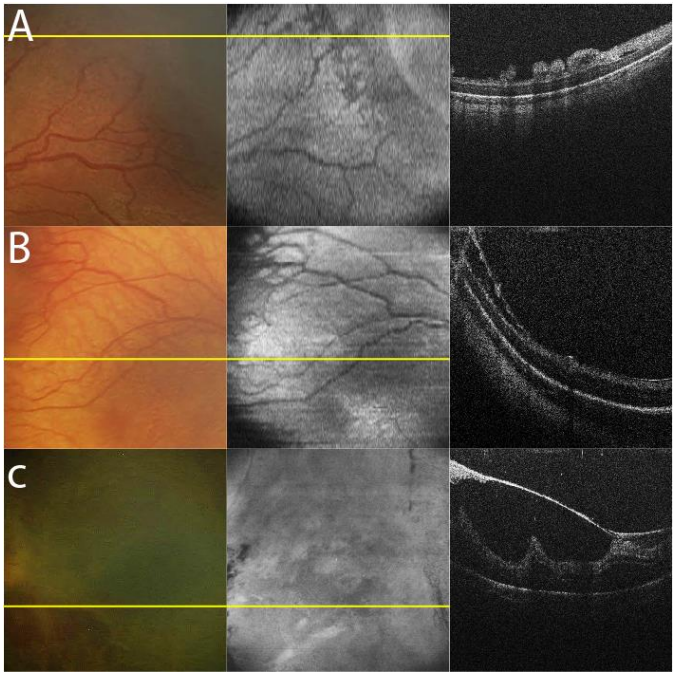
2) indicators of plus disease: axial and lateral retinal vascular tortuosity, hyporeflective vessels, perivascular hyporeflective retinal spaces, perivascular retinal surface elevation [10] (eFigure 3)

4) preretinal tissue of APROP [9] (eFigure 3C)

5) Subdivisions of Stage 3 ROP, mild, moderate and severe

6) Regression of stage 3 ROP and deformation of adjacent retina (eFigure 4)

7) Foveal involvement (Stage 4b) versus foveal sparing (Stage 4a) retinal detachment (eFigures 4B, 4C and 5A))



eFigure 4 In active ROP, OCT imaging reveals preretinal tissue, schisis and retinal traction not visible on color photographs.

In infants clinically described as Stage 3 and 4 ROP and imaged with RetCAM (left column), SDOCT images (center column = retinal view, right column = cross-sectional B-scan view) reveal (A) the preretinal neovascularization at the vascular-avascular junction and thickened avascular inner retina with cystoid space adjacent to the neovascular border: (B) tractional retinoschisis (left half of B-scan) in an eye with regressed stage 3 ROP and clinically-determined

as stage 4A ROP; and (C) preretinal fibrovascular tissue and condensed hyaloid with severe vitreoretinal traction without retinal detachment in an eye with clinically-determined stage 4B ROP.

Additional OCT features that may reflect disease activity:

1) Focal hyperreflective sites (exudates)
2) Retinoschisis which is usually associated with vitreous traction such as in areas of active and regressed Stage 3 disease (Figure 5B)

(b) The following SDOCT structural ROP outcomes would be useful in the early and late outcomes. OCT imaging is currently standard for assessment of central retina and peripapillary retinal nerve fiber layer thickness in adults and children and has been demonstrated in preterm infants with ROP [5,11,24,46,47]. Without OCT imaging, assessment in eyes without a good foveal light reflex may miss findings detailed below which would provide useful subdivision of anatomical outcomes.

Optic nerve:

1) retinal nerve fiber layer thickness
2) measurement of hypoplasia
3) optic nerve head deformation (Figure 6C)

Central retina:

1) true retinal detachment in the foveal center, macula or in zone I versus retinoschisis, (Figure 6A)
2) foveal dragging away from or towards the optic nerve head (Figure 6)
3) posterior hyaloidal organization and epiretinal membranes (Figure 5C)

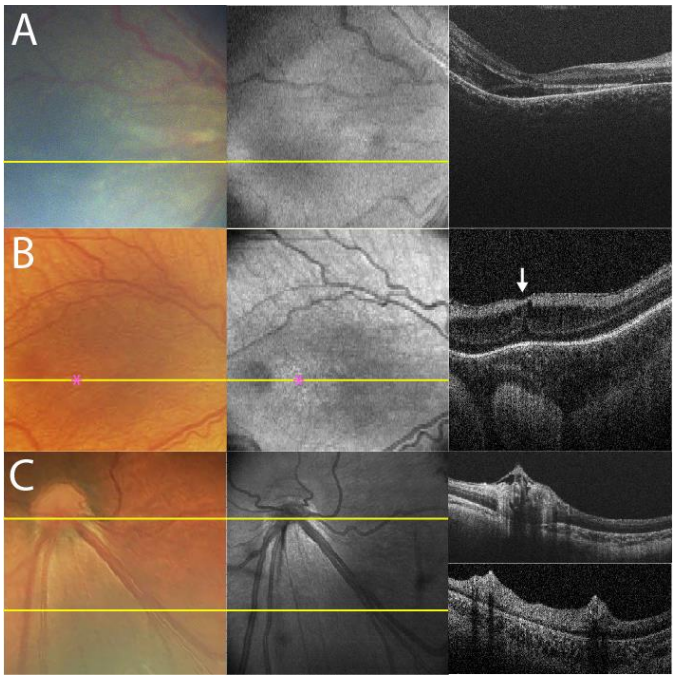
3) posterior retinal fold(s), severity, macular involvement within the fold (Figure 6C)

4) residual preretinal structures

7) shadowing from preretinal structures or blood

9) vitreomacular or vitreoretinal traction (Figure 5B &C)

10) exudates



eFigure 5. OCT of retinal detachment, foveal location and dragging in ROP.

In contrast to color photographs (left column), SDOCT imaging aids in determination of foveal involvement in infant eyes with ROP with the OCT retinal view (center column) for orientation and cross-sectional B-scans (right column) which sum up to create the retinal view. (A) Color photo and OCT retinal view do not differentiate the schisis (left side of the OCT B-scan) from the retinal detachment (Stage 4B ROP, at the center of B-scan). (B) SDOCT cross-sectional image reveals the precise location of the fovea and aids in determination of foveal dragging, foveal cystoid spaces and an epiretinal membrane in an eye with regressed stage 3 ROP. There is also a lack of foveal photoreceptor development based on thickness of the photoreceptor layer and absence of ellipsoid zone at the fovea. (C) OCT cross-sectional image shows elevation of the optic disc as well as retinal folds at the major blood vessels in an eye with stage 4A ROP. The fovea was dragged towards the periphery and into another frame of the imaging.

(c) In preterm-born infants, retinal structure is rapidly evolving. SDOCT images reflect not only the structural changes of the ROP disease processes but also programmed cellular development, migration and apoptosis, a process that continues for years after term birth and which is not able to be characterized on clinical examination [10,48]. While this development may be affected by ROP, it may also be affected by preterm birth, central nervous system events, systemic health and therapeutic interventions.

The following structural outcomes visible on SDOCT include presence of and measurement of the dimensions of features which may reflect the impact of ROP or therapeutics on the retina and retinal development or may be independent of ROP disease. (e.g. eFigure 3 and eFigure 5B)

1) foveal thickness

2) measures of foveal pit development: depth or volume of the foveal pit; ratio of foveal to parafoveal thickness; inner retinal layers and bands at the foveal center and across the macula: nerve fiber layer, ganglion cell layer, inner plexiform layer, inner nuclear layer (eFigure 3)

3) photoreceptor development: photoreceptor layers and bands at the foveal center and across the macula: Henle's layer, outer nuclear layer, external limiting membrane, ellipsoid zone, photoreceptor interdigitation zone (eFigures 3 and 5)

4) cystoid macular structures (eFigures 3 and 5)

5) retinal nerve fiber layer thickness around the optic nerve head

6) retinal pigment epithelium

7) choroidal thickness

8) on OCT-A, retinal vascular pattern of the foveal avascular zone

eAppendix 4. Overview of fluorescein angiography for acute assessment and structural outcomes in ROP

Fluorescein angiographic imaging, although an invasive test, has the potential to provide useful robust data on the extent of the vascularized retina, vascular abnormalities including neovascularization and response to novel treatments in acute phase ROP, and robust short and long-term vascular outcome measures for clinical trials. In many locations where ROP care is provided, there is limited access to fluorescein angiographic imaging technology.

Fluorescein angiography (FA) has been used to image retinal and extraretinal vasculature since the late 1960s in (retrolental fibroplasia and) ROP. FA is an invasive test that requires fluorescein sodium dye within the vasculature and the use of special filters to control the wavelengths of light delivered to the eye (blue light, 465-490 nm) and captured by the retinal camera (520-530 nm) to optimize imaging of the fluorescence. Fluorescein sodium is given in an intravenous injection and pediatric doses are based on body weight. FA imaging at the bedside or during examination under anesthesia can be performed with portable cameras equipped for FA and using many of the techniques described in Appendix A. Injection of the dye discolors the skin and urine and has the potential to result in adverse reactions, and US FDA approved labelling notes that "Pediatric patients have been included in clinical studies. No overall differences in safety or effectiveness have been observed between pediatric and adult patients." (https://www.accessdata.fda.gov/drugsatfda_docs/nda/2008/022186s000_Lbl.pdf) Multiple authors have reported a lack of side effects after intravenous fluorescein use in small series of preterm infants [49]. Fluorescein sodium has also been given orally (off label) for retinal imaging in infants.

Fluorescein sodium remains within the endothelial cell tight junctions of healthy retinal vasculature and on the choroidal side of the retinal pigment epithelium. Fluorescein angiographic images can thus provide information on retinal vascular patterns, avascular or nonperfused areas and leakage from neovascularization, across the posterior pole and in the periphery. Although used in ROP imaging for almost 50 years, FA imaging studies in ROP have been generally been descriptive and from single centers and findings have not been validated through larger prospective studies or across multiple centers. The FA imaging has been utilized later in the course of disease and at greater intervals than photography and retinal examination.

In acute phase ROP, FA has been shown to reveal otherwise unrecognized shunting, areas of capillary nonperfusion, location of the fovea, extraretinal neovascularization, aggressive posterior ROP and the location of the vascular-avascular junction, all of which may be useful in determining disease severity (Figure 1) [22–24]. The contrast of the bright fluorescent vessels or vascular leakage against dark avascular retina, can be particularly helpful for these assessments compared to examination or color photographs alone and especially in eyes with vitreous haze or with a blonde fundus [23,24].

FA has also aided in the assessment of vascular outcomes after ROP treatment, revealing: areas of peripheral nonperfusion, abnormal vascular branching, looping or capillary nonperfusion in the periphery or in the macula, a smaller foveal avascular zone and fluorescein leakage from vessels at the vascular-avascular junction or from extraretinal neovascularization (Figure 2).

While FA imaging may reveal different aspects of ROP disease and the impact of treatment on the retina, it may also reflect retinal development or vascular effects of non-ocular therapies independent of ROP disease.

(a) The following FA findings reflect retinal vascular development and ROP disease activity [22–24,50].

1) foveal center (to estimate zone I, Figure 1)

2) extent of retinal vascularization (to estimate zone)

3) extent of leaking neovascularization for diagnosis of Stage 3 ROP and its subdivisions

4) regression of stage 3 ROP

5) recurrence of neovascularization

6) retinal vascular abnormalities and abnormal fluorescein leakage

(b) The following FA vascular findings would be useful for the early and late outcomes [22,23] and some have been shown to differ between treatments for ROP:

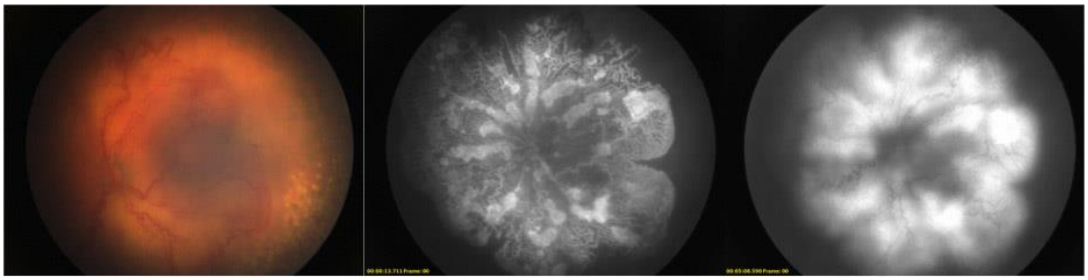
1) extent of retinal vascularization

2) vascular development of the foveal avascular zone

3) location of fovea and retinal dragging

4) retinal vascular abnormalities including nonperfusion and shunt vessels

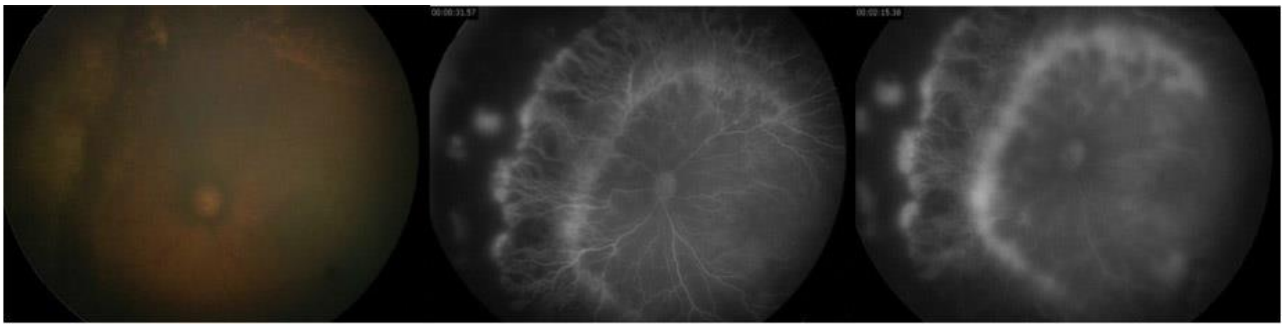
5) retinal pigment epithelial abnormalities and laser scars



eFigure 6. Tortuous retinal vessels of plus disease with FA.

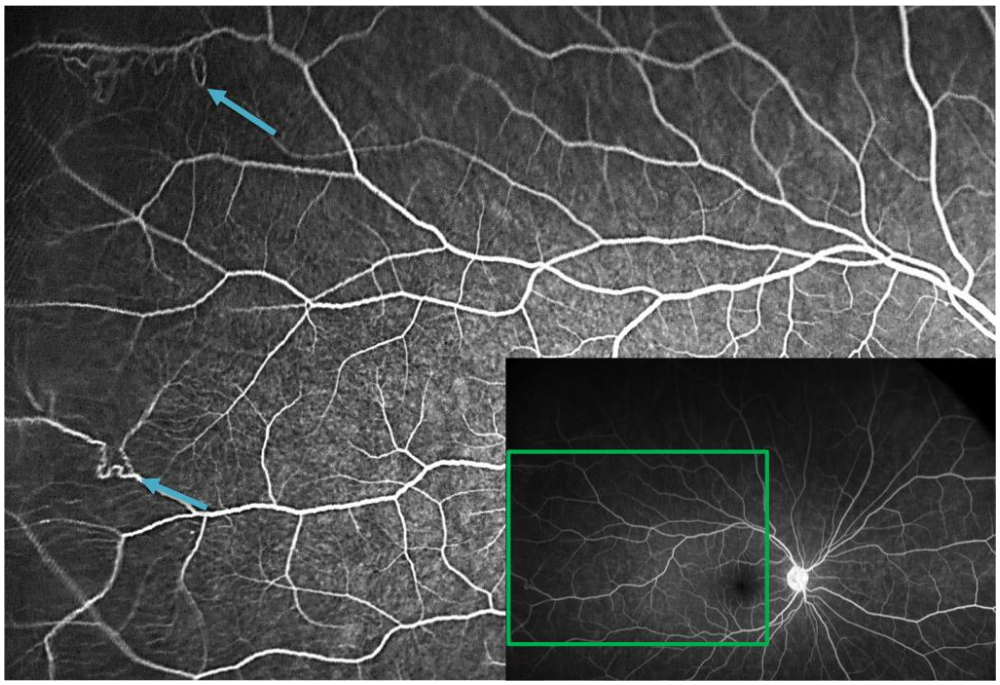
In an extremely low birthweight infant with ROP, the color photograph (left) documents tortuous vessels of plus disease with shunt vessels and neovascularization, lack of a discernable foveal configuration, and laser scars in the periphery. The fluorescein angiogram (right) reveals

profound abnormalities of all retinal vasculature, with abnormal vascular patterns and branching and patchy areas of nonperfusion (dark areas on the fluorescein angiogram (FA) across the vascularized retina, an avascular periphery and extensive leaking neovascularization across the posterior pole (hyperfluorescent patches in middle frame with late leakage on right frame). Such findings were described by Yokoi in aggressive posterior ROP [50].



eFigure 7. FA reveals retinal vascular details not seen in photographs

In early outcomes assessment, in this case at 43 weeks postmenstrual age, the fluorescein angiogram (right) reveals vascular details not apparent on the color photograph (left). On the color photograph, the peripheral retinal vasculature is tortuous over the laser scars and there is pale preretinal tissue along the posterior border of the laser scars, but the fluorescein angiogram reveals a large arc of regressed neovascularization (with late leakage) midway between the disc and the hyperfluorescent leaking vessels at the margin of the vascularized retina, leaking vessels within the otherwise avascular lasered peripheral retina, and the lack of a well-formed foveal avascular zone.



eFigure 8. Abnormal peripheral vascular anomalies seen only with FA

In assessment of late outcomes, wide-field fluorescein angiography reveals details such as abnormal peripheral vascular loops, which were not appreciated on fundus examination but were present in the temporal periphery of both eyes, in this young teenager with a history of premature birth.