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Suspended Scattering Particles in Motion: A Novel Feature of OCT Angiography in Exudative Maculopathies

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Abstract

Objective—To characterize features of extra-vascular optical coherence tomography angiography (OCTA) signals corresponding to hyperreflective intraretinal fluid across various exudative maculopathies.

Design—Multicenter, retrospective, observational study.

Participants—Eyes with various forms of exudative maculopathy including diabetic retinopathy (DR), retinal vein occlusion (RVO), and neovascular-age related macular degeneration (nvAMD).

Methods—Patients with extra-vascular OCTA signal identified on *en face* OCTA images were included in this study. This signal was readily distinguishable from projection artifacts. The regions with the extra-vascular motion signal on OCTA were named “Suspended Scattering Particles in Motion (SSPiM).” Depth-encoded, color, *en face* OCTA images (3mm × 3mm) centered on the fovea and their corresponding structural OCT scans were used to quantify features

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of SSPiM and its corresponding hyperreflective fluid. Longitudinal data were collected when available.

Main outcome measures—Anatomic location, the association with hyperreflective material, changes in location and appearance of SSPiM over time, and replication of SSPiM OCTA signal in an *in vitro* phantom.

Results—Seventy-six eyes in 62 patients with various forms of exudative maculopathy were evaluated; 60 eyes with DR, 9 eyes with RVO, and 5 eyes nvAMD, 1 eye with macroaneurysm, and 1 eye with radiation retinopathy. Intraretinal accumulations of fluid with increased OCT signal intensity corresponded to regions of SSPiM in several exudative maculopathies. An *in vitro* phantom model demonstrates that particulate matter in suspension can generate similar OCTA signal. SSPiM showed an anatomic preference for vascular-avascular junctions. The hyperreflective fluid corresponding to SSPiM appeared more frequently in Henle's fiber layer (HFL) than the inner nuclear layer (INL) and was highly associated with hyperreflective material (HRM) found bordering the fluid. In five of eight longitudinal cases, the resolution of SSPiM resulted in the formation of confluent HRM. Clinically, this appeared as hard exudate on funduscopy images.

Conclusions—Clinical data suggest that SSPiM is a novel imaging feature of retinal vascular diseases that was not appreciated prior to the use of OCTA. We characterized several novel features of SSPiM and demonstrated that at least in some cases it resolves with residual hard exudate.

Introduction

Optical Coherence Tomography Angiography (OCTA) is an emerging imaging modality that uses change or decorrelation in the optical coherence tomography (OCT) signal to detect motion in biological tissue. OCTA devices can construct detailed maps of the retinal microvasculature by comparing signals from objects in motion such as red blood cells against more static structures, such as photoreceptors and neurons.

In contrast to the steady flow of fluid and red blood cells in retinal vessels, static deposits of intraretinal fluid would presumably be devoid of an OCTA decorrelation signal, similar to other non-vascular tissues. However, in our previous studies of subjects with diabetic retinopathy (DR) and retinal vein occlusion (RVO), OCTA detected a decorrelation signal within some intraretinal collections of fluid.^{1,2} These areas of increased OCTA signal generally appeared as oval or circular regions of fluid on both cross-sectional and *en face* images. This decorrelation signal correlated with the hyperreflective fluid seen on the cross-sectional structural OCT that was previously distinguished from hyporeflective fluid by Liang et al.³

We previously hypothesized that this extra-vascular OCTA signal was the result of particulate matter suspended in intraretinal fluid and coined the term “intraretinal fluid associated with hard exudate” because of the association with hard exudates in our previous cases.^{1,2} Subsequently, we have observed this finding in the absence of clinical hard exudates. Therefore, we have chosen a more descriptive term for this extra-vascular OCTA

signal, “suspended scattering particles in motion” or SSPiM.⁴ However, the prevalence of this finding, its etiology, and its clinical relevance have not been adequately studied.

The purpose of the present study is to further characterize the anatomic, temporal, and physical properties of SSPiM and demonstrate its prevalence across several exudative maculopathies.

Materials and Methods

This was a multicenter, retrospective, observational study that was conducted across six tertiary care retina centers encompassing both private and academic practices. All centers had IRB approval for general OCTA imaging studies that involve retrospective analysis of images obtained from subjects, which applies to the present study. Each site performed a retrospective review of OCTA images from any subject imaged over the past 24 months to identify subjects with intraretinal fluid pockets or cysts that demonstrated extra-vascular OCTA signal intensity corresponding to SSPiM as described originally.^{1,2} Special attention was focused on subjects with a history of DR and RVO, but any subject with SSPiM identified on *en face* OCTA images was included in this study. Therefore, the main inclusion criterion for this study was the presence of SSPiM. Additional criteria included sufficient image quality of the OCTA images. Specifically, a signal strength index of at least 8/10 for the Zeiss systems (Carl Zeiss Meditec Inc; Dublin, CA) and at least 55 for the Optovue system (Optovue Inc; Fremont, CA) was required. Among our six sites, the total number of OCTA images reviewed included approximately 350 of DR, 120 of RVO, and 80 of AMD. To determine the approximate frequency with which SSPiM occurs in cases with macular edema, we retrospectively reviewed all the OCTA images in our database at USC and identified those with macular edema apparent on corresponding structural OCT. We then calculated the proportion with SSPiM in these cases for each disease.

All eyes were imaged with a commercial spectral-domain OCTA (SD-OCTA; AngioPlex™ Carl Zeiss Meditec, Dublin, CA or AngioVue™ Optovue, Fremont, CA) or with a swept-source device (SS-OCTA; PlexElite™ Carl Zeiss Meditec). The AngioPlex uses an optical microangiography algorithm, with an 840nm center wavelength, at a speed of 68,000 A-scans per second. The AngioVue uses a split-spectrum amplitude decorrelation algorithm, with an 850nm center wavelength, at a speed of 70,000 A-scans per second. The PlexElite uses swept-source OCT, with a 1060nm center wavelength, at a speed of 100,000 A-scans per second. Of the 76 eyes in our study, 61 were imaged using AngioPlex, 10 using Optovue, and five using PlexElite. All OCTA images consisted of at least one 3mm × 3mm image centered on the fovea and were either viewed as greyscale or color depth-encoded images. For patients with longitudinal data representing more than one visit over a period of months, OCTA images were collected. Clinical data including age, gender, best corrected visual acuity, history of hypertension, history of hyperlipidemia, and history of treatment with vascular endothelial growth factor (VEGF) inhibitors and/or laser were collected for every subject.

Several anatomic features of SSPiM were analyzed using the 3mm × 3mm depth-encoded *en face* color flow images and a single, representative, cross-sectional OCT B-scan overlaid

with the OCTA flow information through areas of SSPiM. We also reviewed the full-thickness structural *en face* retinal images.

In order to model the possible etiology of SSPiM an *in vitro* phantom was created using a solidified background of 0.4% TiO₂ and 25% gelatin in water. This concentration of TiO₂ is completely solid at room temperature and has no particulate material that is freely diffusible. Small wells were created within the phantom and were filled 1% gelatin - 1% intralipid solution to simulate small scattering particles. Intralipid is an aqueous suspension of lipid droplets that can be diluted to desired concentrations. This solution was used to approximate the particulate material within intraretinal fluid pockets that occur in the retina. A control suspension was made of 2% pure gelatin with no intralipid. The phantom was imaged using a custom made 100kHz OCT system. The light source has a central wavelength of 850nm and spectrum bandwidth of 45 nm.

Results

We report the results from a total of 76 eyes in 62 subjects with exudative maculopathy including 60 eyes with diabetic macular edema; four with mild non-proliferative diabetic retinopathy (NPDR), 22 with moderate NDPR, seven with severe NDPR, and 27 with proliferative diabetic retinopathy (PDR). There were nine eyes with RVO, including seven with branch RVO (BRVO), one with a central RVO (CRVO), and one with a hemi-central RVO. Five eyes had neovascular age-related macular degeneration (nvAMD), one eye had a retinal arterial macroaneurysm, and one eye had radiation retinopathy. Patient demographics and clinical features are displayed in Table 1. All patients were noted to have an extra-vascular OCTA signal that we identified as SSPiM. Regions of SSPiM in all *en face* images in this study correlated with hyperreflective fluid on structural OCT in all cases. Likewise, any hyperreflective signal on structural OCT that corresponded with location of intraretinal fluid always had accompanying SSPiM signal. The prevalence of SSPiM in macular edema was determined by reviewing all OCTA images in our database at one site (USC). In total, 79 of 151 eyes with DR, 25 of 62 with RVO, and 21 of 75 of eyes with nvAMD had macular edema. Among the diabetic eyes with macular edema, 22 of 79 (27.8%) had SSPiM. More specifically, zero of 16 with mild DR, 10 of 15 (66.7%) with moderate DR, five of 19 (26.3%) with severe DR, and seven of 29 (24.1%) with PDR. For RVO with macular edema, four of 25 (16.0%) had SSPiM, and for nvAMD with macular edema, five of 21 (23.8%) had SSPiM. Representative examples of SSPiM found in moderate and severe NDPR, PDR, RVO, and nvAMD are shown in Figure 1. SSPiM was associated with varying degrees of hyperreflective material (HRM) on structural OCT that bordered the hyperreflective fluid. The HRM was most often present as well-defined pinpoint areas of hyperreflectivity. HRM are seen clearly bordering hyperreflective fluid in Figure 1a, 1d, and 1e. Overall, 64.5% of hyperreflective fluid regions were associated with at least one area of HRM within a single cross-sectional B-scan (Table 2). We also performed *en face* structural analysis in a subset of our data, and found that all cases had at least one area of HRM along the border of hyperreflective fluid.

Several additional anatomic characteristics of SSPiM are also displayed in Table 2. Of note, SSPiM always appeared to be present at the edge or border of a vascular plexus at a

vascular-avascular junction. Vascular-avascular junctions were defined as regions where terminal capillaries were noted including the foveal avascular zone (FAZ) and the outer plexiform layer (OPL). Overall, all of our cases displayed SSPiM at one of these junctions. More specifically, 85.5% of our cases displayed SSPiM that either directly bordered or crossed the FAZ in the 3mm × 3mm images. Likewise, nearly all of our cross-sectional and *en face* OCT and OCTA images illustrated some portion of hyperreflective fluid directly bordering or crossing the OPL with rare exception. Additionally, the hyperreflective fluid corresponding to SSPiM illustrated an anatomic preference for Henle's fiber layer (HFL). When looking at single cross-sections of SSPiM for all our cases, 88.2% displayed hyperreflective fluid present in HFL, whereas only 19.7% had such fluid present in the inner nuclear layer (INL). A preference for deeper retinal layers can also be appreciated in *en face* visualizations of SSPiM at different depths in Supplemental Figure 1 (available at www.aaojournal.org). In no cases did we observe any SSPiM that corresponded to subretinal fluid.

SSPiM could also be found more peripherally or directly centered within the FAZ. In certain cases, SSPiM extended outside of the 3mm × 3mm window and could be further visualized on 6mm × 6mm images. The size of SSPiM varied from small irregular circular or oval regions to larger, more confluent areas. Several distinct areas of SSPiM were often present in the same image.

We also explored the longitudinal behavior of SSPiM for a series of eight cases with more than one follow-up visit several months apart when OCTA data were available. All subjects in this group were receiving anti-VEGF therapy. In five of the eight cases (3 PDR, 1 severe NDPR, and 1 BRVO), the SSPiM largely resolved with the formation of coalesced areas of HRM seen on B-scan, a process that took an average of 172 ± 35 days. Corresponding funduscopy images were available in two of these (severe DR and BRVO) both of which demonstrated hard exudate corresponding to the area of coalesced HRM. The remaining three of the eight cases of SSPiM (moderate DR, severe DR, and AMD) resolved without the coalescence of HRM, which took an average of 140 ± 15 days. Figure 2 illustrates the case of SSPiM in severe DR that resolved to form hard exudate. Figure 2 also illustrates a direct comparison of *hyper*reflective fluid with accompanying SSPiM and *hypo*reflective with no accompanying SSPiM overtime in the same eye. Overtime, the hyperreflective pocket begins to develop foci of HRM along its periphery. As the pocket of fluid resolves the foci appear to congregate to form more confluent HRM, and the accompanying area of SSPiM begins to disappear (Figure 2A–C). The more confluent areas of HRM could be visualized clinically as hard exudate (Figure 2D–F). In contrast, the hyporefective pocket persists overtime with no development of HRM or hard exudate (Figure 2A–C).

We hypothesize that the origin of SSPiM signal was decorrelation signal from the Brownian motion of particles (lipoproteinaceous debris) within the pockets of intraretinal fluid. In order to test this hypothesis, an *in vitro* phantom using intralipid particles was created to mimic the conditions under which SSPiM signal may occur. Specifically, intralipid particles in solution were suspended in wells made from a solidified background of 0.4% TiO₂ and 25% gelatin in water. In contrast to the TiO₂ containing background material which is completely solid at room temperature and has no particulate material that is freely diffusible,

the intralipid solution (1% gelatin - 1% intralipid) solution demonstrated significant SSPiM signal (Figure 3). A control well containing pure gelatin had minimal or no detectable SSPiM signal similar to the TiO₂ background.

Discussion

The present study characterized an extra-vascular OCTA signal that corresponded to hyperreflective fluid in several exudative maculopathies. As OCTA detects motion in biological tissue, we hypothesized this fluid has suspended particulate matter, hence the term “suspended scattering particles in motion” (SSPiM). SSPiM was identified in all 76 eyes with exudative maculopathy, 60 of which were diabetic. Interestingly, only four of our diabetic cases were categorized as mild, suggesting that SSPiM may develop primarily in more advanced disease. Given that most of our data is comprised of cases of DR, our conclusions on the characteristics of SSPiM cannot be generalized with certainty to all exudative maculopathies. However, similar trends were observed across several diseases (RVO, nvAMD, radiation retinopathy and macroaneurysm), as seen in Table 2.

The clear anatomic preference of SSPiM for HFL was one of the most consistent features, with 88.2% of our cases illustrating evidence of hyperreflective fluid present in this layer. SSPiM in the INL was far less common with an overall prevalence of 19.7%. Of note, the presence of hyperreflective fluid in HFL and the INL was not mutually exclusive. Our cases with diffuse edema displayed fluid in multiple layers in any given cross-section. This is consistent with Byeon et al.’s finding that more diffuse leakage in DME results in fluid deposition in the INL and secondarily in the OPL/ONL, which includes HFL.⁵

We also observed that all of our SSPiM displayed an anatomic preference for vascular-avascular junctions, with 85.5% of cases illustrating SSPiM crossing or bordering the FAZ in an *en face* view. If areas of SSPiM were randomly distributed over a 3mm × 3mm window, larger areas would have a greater probability of bordering or crossing the FAZ. However, we noted that even smaller, sometimes minute regions of SSPiM had a tendency to localize at this junction. Furthermore, all of our cases displayed hyperreflective fluid bordering or crossing the outer plexiform layer, which was true for fluid located in either HFL or the INL.

Another finding in our study was the anatomical association of HRM with hyperreflective fluid. The typical appearance of these HRM can be appreciated in Figure 1. Gelman et al. has similarly described hyperreflective “dots” on the inner border of cystoid spaces in various exudative maculopathies, including neovascular AMD and RVO.⁶ Ultimately, there is still a lack of consensus regarding the origin and significance of all HRM in the retina. One study recently evaluated characteristics of these hyperreflective “spots” and compared them with features on *en face* images.⁷ They suggested that when larger than 30µm, these spots may represent hard exudate in the outer retina. For DME patients, Bolz et al. suggests that HRM may represent subclinical extravasation of lipoproteins and/or proteins, secondary to breakdown of the blood retinal barrier.⁸ Of particular interest in this study, Bolz et al. also noted that accumulations of HRM tend to occur at the outer retina at the border of the ONL and in the OPL, which includes HFL. These could be visualized as hard exudate. Similar

observations have also been reported in RVO.⁹ These previous findings parallel our results showing that hyperreflective fluid, which was associated with HRM, also had an anatomic preference for the HFL.

For five of our eight longitudinal cases, we observed that as SSPiM resolved overtime the HRM increased in number and eventually coalesced to form larger areas of HRM. In both cases where corresponding fundusoscopic images were available, the coalesced HRM could be visualized clinically as hard exudate, as seen in Figure 2. However, SSPiM could also resolve without the formation of HRM and hard exudate, which occurred in the remaining three of the eight cases. Furthermore, this process did not necessarily occur throughout the entire area of SSPiM for cases where it was observed. Some cases illustrated a stark contrast between the evolution of hyperreflective and hyporefective fluid, as seen in Figure 2. Such contrast suggests that the higher concentrations of particulate matter suspended in hyperreflective fluid may contribute to the more rapid development and confluence of HRM compared to hyporefective fluid. Of note, all the patients in our longitudinal cases were undergoing anti-VEGF therapy. Consistent with our findings is a previous report by Pemp et al. that showed DME patients with confluent hyperreflective structures being treated with anti-VEGF, which was then accompanied by an enlargement of hard exudates overtime.¹⁰

Overall, we have a high degree of confidence that SSPiM signal is derived from particles in motion. If the cause of SSPiM were some form of stationary particles that change their reflectance overtime, we would expect to see greater variance in signal intensity on structural OCT in corresponding areas, which was not observed (Supplemental Figure 1A). Furthermore, it is not the case that hard exudate or other objects in these images have variable reflectance that is causing the SSPiM signal. The absence of flow signal from the region of coalesced HRM and hard exudate in Figure 2C confirms this observation. To further confirm whether the motion of suspended particles could give rise to observable OCTA signal, three of the authors (ZC, QZ, RKW) created a phantom *in vitro* model to demonstrate that intralipid particles suspended at a concentration of 1% in a solution of 1% gelatin clearly demonstrated an OCTA signal consistent with SSPiM (Figure 3A). This finding confirms that at least lipid aggregates alone can generate such a signal, which may represent a key component of the suspended particles in SSPiM. It is likely that lipoprotein aggregates demonstrate similar optical behavior in OCTA imaging, although this remains to be shown.

While our understanding of the specific etiology of hyperreflective fluid that gives rise to SSPiM is speculative, the breakdown of the blood-retinal barrier is the primary cause of macular edema in DR, RVO, AMD, and uveitis.¹¹ The degree of BRB damage and vascular permeability may reflect the type and amount of exudate that enters the intraretinal space, with more advanced breakdown presumably giving rise to more particles and hence more hyperreflectivity. Although it is generally assumed that the fluid is in the interstitial space, it should be noted that the exact location of this fluid (intracellular versus extracellular) is not known. Throughout this paper we have made a distinction between hyperreflective fluid and hyporefective fluid. However, there is a spectrum between the two types of fluid, which likely represents varying concentrations of suspended particles. Even hyporefective fluid that does not have an associated OCTA signal certainly contains some degree of particulate

matter such as extravasated protein, as would be necessary for Starling forces to induce edema.¹² In diabetic retinopathy in particular, Müller glia are believed to be a major contributor to neovascularization, vascular leakage, and breakdown of the BRB, primarily through the production of VEGF.^{13,14} Indeed, they may also play a critical role in other diseases with macular edema including RVO and nvAMD, where VEGF inhibitors can be used to treat edema in such cases. Another possible cause of SSPiM is leakage from microaneurysms. In DME, Byeon et al. correlated microaneurysms with the development of fluid in the OPL/ONL, where the majority of hyperreflective fluid in our study was found. Hasegawa et al. demonstrated an association between microaneurysms in the deep capillary plexus and the location of DME, and also found that density of these microaneurysms was associated the type of DME.¹⁵ The leakage of microaneurysms could theoretically cause exudation of larger quantities of particulate debris that become suspended in pockets of fluid, and subsequently give rise to OCTA signal. It is also possible that multiple pathologic processes work in concert.

The etiology of HRM in relation to SSPiM is another consideration. Taarnhøj et al. and Kashani et al. previously described the kinetics of lipoproteinaceous debris in the retina, suggesting that resorption of water and salt by the retinal pigment epithelium and intraretinal capillaries, accompanied by a lack of mechanism to resorb macromolecules, leads to precipitation of these macromolecules as their concentration increases.^{16,17} It has been shown that the optical density of subretinal fluid increases over time after retinal detachment repair and this suggests an increasing clearance of water versus particulate debris in the subretinal space by RPE.¹⁷ Interestingly, the lack of SSPiM findings in the subretinal space in the current study of exudative retinopathies suggests that particulate debris may be cleared more efficiently from the subretinal space than the intraretinal space. A more extensive study of subretinal fluid containing pathology is warranted to address this possibility. Regarding the HRM associated with SSPiM, it is possible that the environment and configuration of vascular-avascular junctions lends itself to a unique balance of leakage and resorption, leading to greater precipitation of HRM. Alternately, phagocytic activity may be hindered at these junctions, leading to persistence and accumulation of any material that would normally be eliminated. It remains unclear whether the HRM represents intracellular or extracellular debris, or both.

It is unclear precisely what type of particles present in intraretinal fluid are detected by OCTA *in vivo*, although it is likely a combination of lipid and proteinaceous macromolecules. While suspended lipid particles alone can generate an OCTA signal per our phantom, our current thought on the composition of hyperreflective material and hard exudates, and the association of these entities with SSPiM, suggests that lipids may indeed be a contributor to the particles detected *in vivo* in this study. In 1996 the Early Treatment Diabetic Retinopathy Study demonstrated that elevated serum lipid levels were associated with increased retinal hard exudates in diabetic retinopathy.¹⁸ Similarly, regression of these exudates has been observed following reduction of serum lipid levels.¹⁹ Cusick et al. also provided histological evidence of concentrations of the 2 primary components of LDL. Apolipoprotein-B (a major peripheral component of LDL) stained along vessels and cholesterol ester (a major core component of LDL) stained with a more diffuse pattern, suggesting possible extravasation of LDL core components. Regarding HRM, one recent

study correlated higher total and LDL cholesterol with HRM on SD-OCT, in addition to greater macular volume.²⁰ Considering these studies in light of our clinical and phantom data, it seems plausible that extravasated lipids may both be a contributor to SSPiM and subsequently a contributor to the formation of associated HRM and hard exudates.

The major limitation in this study is the small sample size for cases of SSPiM in non-DR exudative maculopathy. Furthermore, although we had several cases in which the resolution of SSPiM was associated with the development of hard exudate, our limited pool of longitudinal data made it impractical to adequately assess the frequency with which this occurs, particularly in non-DR cases. In addition to increasing sample size, future studies may also aim to characterize SSPiM in additional maculopathies not described in our study, such as uveitis.

Overall, our conclusions in this study can be summarized as follows: (1) extra-vascular OCTA signal corresponding to hyperreflective fluid is caused by suspended scattering particles in motion, and we therefore refer to this signal as “SSPiM.” (2) SSPiM can be found in various forms of exudative maculopathy. (3) SSPiM has an anatomic preference for vascular-avascular junctions and is more commonly found in HFL than the INL. (4) in at least some cases the resolution of SSPiM is associated with foci of HRM that can coalesce and be visualized as hard exudate clinically. The latter two conclusions may be true in general for exudative maculopathy, but can be applied with greater confidence to DR given our sample sizes. The clinical utility of these findings requires further study. However, clinicians who have been puzzled by unusual, extra-vascular signal on OCTA *en face* images may now appreciate it as SSPiM. Furthermore, they can be aware that in some cases SSPiM may resolve and be associated with hard exudate.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations and acronyms

BRVO	branch retinal vein occlusion
DR	diabetic retinopathy
HRM	hyperreflective material

INL	inner nuclear layer
NPDR	non-proliferative diabetic retinopathy
nvAMD	neovascular age related macular degeneration
PDR	proliferative diabetic retinopathy
RVO	retinal vein occlusion
SD-OCTA	spectral-domain optical coherence tomography angiography
SSPiM	suspended scattering particles in motion
OCT	optical coherence tomography
OCTA	optical coherence tomography angiography
ONL	outer nuclear layer
OPL	outer plexiform layer

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Figure 1.

SSPiM and corresponding hyperreflective intraretinal fluid seen in various exudative maculopathies. All 3mm × 3mm depth-encoded color *en face* OCTA images with accompanying structural OCTs were captured using an SD-OCT (AngioPlex or Optovue) or SS-OCT (PlexElite) device. On depth-encoded images red corresponds to the superficial retinal layer, and green to the deep retinal layer. Structural OCTs above each *en face* image have superimposed OCTA signal (in red) to better distinguish the hyperreflective fluid. All yellow arrows point to hyperreflective fluid on structural OCTs and SSPiM on the *en face* images. (A) 53-year-old female with moderate NPDR and history of anti-VEGF treatment. Hyperreflective fluid is seen on the enlarged structural OCT in HFL and is bordered by HRM. The superficial foci of HRM is at the junction of HFL and the OPL whereas the deep foci of HRM rest above the ONL. The SSPiM on the *en face* image corresponding to the hyperreflective fluid begins at the border of the FAZ and extends peripherally (AngioPlex). (B) 68-year-old female with a moderate NPDR and history of anti-VEGF treatment. A central area of SSPiM corresponds with hyperreflective fluid with flow signal (AngioVue). (C) 70-year-old male with severe NPDR. A central area of hyperreflective fluid is accompanied by centrally located SSPiM. A smaller area of fluid on OCT can be appreciated in the ONL with corresponding SSPiM at the 3 o'clock border of the FAZ (AngioPlex). (D) 67-year-old female with PDR and history of PRP laser treatment. Hyperreflective fluid in HFL has corresponding SSPiM near the border of the FAZ (AngioPlex). (E) 67-year-old female with moderate branched retinal vein occlusion (BRVO) and known history of anti-VEGF treatment. A small pocket of hyperreflective fluid in HFL is bordered by foci of hyperreflective material. The corresponding SSPiM also exists as a small region on the border of the FAZ. There is additional SSPiM present in the *en face* image that falls outside of the structural OCT cross-section (AngioPlex). (F) 58-year-old female with hemi-retinal vein occlusion (HRVO). SSPiM is largely present in area affected

by occlusion with corresponding hyperreflective fluid visible on structural OCT (PlexElite). (G) 77-year-old female with neovascular AMD and history of anti-VEGF treatment. The macula is slightly off-center in the 3mm × 3mm window with extensive SSPiM filling in the FAZ and its borders. A ring of HRM can be seen forming around the corresponding hyperreflective fluid on B-scan. (H) 65-year-old male with neovascular AMD and history of anti-VEGF treatment. A larger area of hyperreflective fluid is bordered by foci of hyperreflective material which appear to coalesce near the right side of the structural OCT image. The corresponding SSPiM is a large area on the border of the FAZ (AngioPlex).

Figure 2.

Resolution of SSPiM associated with formation of hard exudate. This is a case of severe non-proliferative diabetic retinopathy in a 56-year-old-male followed over 7 months. His best corrected visual acuity at t = 0 months was 20/40 and at t = 7 months was 20/30. The patient has a history of focal laser treatment and multiple anti-VEGF injections. He received 3 injections during this 7-month period: aflibercept at t = 0 and 6 months, and dexamethasone at t = 3.5 months. The 3×3 depth-encoded, color, *en face* OCTA images with accompanying structural OCTs (A–C) were captured using an SD-OCT device. (A) Initially at t = 0 months there is a large area of hyperreflective fluid seen on structural OCT with corresponding SSPiM (yellow arrows). The green line represents the cross-section of the *en face* image where the structural OCT was obtained. This was taken in a similar location in each image using vascular landmarks. The SSPiM can be appreciated as red or green corresponding to depth. There is also a large hyporeflexive pocket present in the structural OCT with no corresponding SSPiM (white arrows). (B) At t = 3.5 months there is an increasing number of foci of hyperreflective material that are beginning to coalesce as seen on structural OCT. There appears to be a decrease in SSPiM in areas corresponding to the development of hyperreflective material. (C) The SSPiM is mostly resolved with the exception of some signal in the center of the fovea. Abundant, confluent hyperreflective material is seen on structural OCT. The hyporeflexive pocket has persisted with no corresponding SSPiM and no discernable development of hyperreflective material. (D) At t

= 7 months the confluent hyperreflective material can be seen clinically as hard exudate on color fundus photography. (E–F) The hard exudate seen clinically corresponds to the area of hyperreflective material, as seen on Spectralis OCT. Of note, only a portion of the SSPiM on the *en face* image resolved into hard exudate clinically. For instance, the SSPiM near the 9 o'clock border of the 3mm × 3mm image resolved with no formation of hard exudate.

Figure 3.

In vitro phantom of suspended particles imaged by OCTA. (A) Schematic of phantom with suspended particles in gel-like medium (purple) set in a solidified background composed of 0.4% TiO₂ particles and 25% gelatin. OCTA signal can be viewed in both cross-sectional (single B-scan) and en-face views. (B) OCTA signal is present when imaging 1% intralipid suspended in 1% gelatin solution in both cross-section (B-scan) and in en-face view. In contrast, there is no OCTA signal when imaging the control suspension, composed of pure gelatin with 0% intralipid or the background containing the solid TiO₂.

Table 1

Clinical and demographic features of study population

	Total*	Moderate NDPR	Severe NDPR	PDR	RVO	nvAMD
No. of subjects	62	16	6	24	9	4
No. of eyes	76	22	7	27	9	5
Male : Female	38 : 24	9 : 7	5 : 1	18 : 6	3 : 6	3 : 1
Age, mean (SD), y	57.3 (14.2)	62.8 (11.0)	49.0 (14.6)	51.4 (13.1)	63.9 (9.52)	74 (10.8)
Age range	23 – 88	35 – 77	27 – 70	23 – 72	38 – 75	65 – 88
BCVA, LogMAR	0.28	0.21	0.16	0.27	0.50	0.47
Hypertlipidemia, %	52.1 ^a	75.0 ^b	80.0 ^c	25.0 ^d	33.3	60.0
Hypertension, %	64.0 ^e	57.9 ^f	85.7	55.0 ^g	66.7	60.0
Anti-VEGF tx, %	68.4	63.6	85.7	63.0	66.7	100
Laser tx, %	42.1	18.2	42.9	77.8	22.2	0
CRT, mean, μ m	342	308	375	332	333	450

BCVA = best corrected visual acuity; VEGF = vascular endothelial growth factor; CRT = central retinal thickness. Best corrected visual acuity was collected from date of OCTA imaging. Anti-VEGF tx and Laser tx refer to any past treatments prior to OCTA imaging date. Subjects considered to have prior history of hypertension and hypertension if conditions themselves or medications used to treat them were present in medical records. Data on age and gender reflects number of subjects, all other data reflects number of eyes. Patient data not available in

^a28 cases,

^b10 cases,

^c2 cases,

^d15 cases,

^e12 cases,

^f3 cases,

^g6 cases.

* Patients with mild non-proliferative diabetic retinopathy (3 subjects, 4 eyes), radiation retinopathy (1 eye), and macroaneurysm (1 eye) are not shown separately in table but are included in the total.

Table 2

Anatomic features of SSPiM and hyperreflective intraretinal fluid

	Total* n = 76 eyes	Moderate NPDR n = 22	Severe NPDR n = 7	PDR n = 27	RVO n = 9	nvAMD n = 5
SSPiM at vascular-avascular junction, %	100 n = 76	100 n = 22	100 n = 7	100 n = 27	100 n = 9	100 n = 5
SSPiM associated with hyperreflective material, %	64.5 n = 49	81.8 n = 18	57.1 n = 4	48.1 n = 13	44.4 n = 4	80.0 n = 4
Hyperreflective fluid in HFL, %	88.2 n = 67	95.5 n = 21	85.7 n = 6	92.6 n = 25	88.9 n = 8	100 n = 5
Hyperreflective fluid in INL, %	19.7 n = 15	18.2 n = 4	42.9 n = 3	22.2 n = 6	11.1 n = 1	20.0 n = 1

SSPiM = suspended scattering particles in motion; HFL = Henle's Fiber Layer; INL = inner nuclear layer.

* Patients with mild non-proliferative diabetic retinopathy (3 subjects, 4 eyes), radiation retinopathy (1 eye), and macroaneurysm (1 eye) are not shown separately in table but are included in the total.