Statistical analysis plan (SAP)

Section 1: Administrative information

1.1	Title and trial registration number	The Immediate effect of Defocus incorporated multiple segment spectacle lenses (DIMS) On the Choroid Thickness using Swep Source-OCT on children. The TIDOCT study. ClinicalTrial.gov: NCT06234189
1.2	Names, affiliations and roles of SAP contributors	Lou-Ann C. Andersen, MD, PhD-student Research Unit for the Department of Ophthalmology, University Hospital of Southern Denmark, Lillebaelt Hospital, Vejle, Denmark; University of Southern Denmark, Odense, Denmark Hakim Dalibey, MD Research Unit for the Department of Ophthalmology, University Hospital of Southern Denmark, Lillebaelt Hospital, Vejle, Denmark; University of Southern Denmark, Odense, Denmark Trine M. Jakobsen, MD, PhD, Post.doc Research Unit for the Department of Ophthalmology, University Hospital of Southern Denmark, Lillebaelt Hospital, Vejle, Denmark; University of Southern Denmark, Odense, Denmark Flemming Møller, MD, Consultant, PhD, DMsc, Assoc. Prof. Research Unit for the Department of Ophthalmology, University Hospital of Southern Denmark, Lillebaelt Hospital, Vejle, Denmark; University of Southern Denmark, Odense, Denmark
1.3	Principal investigator/ project lead	Lou-Ann C. Andersen, MD, PhD-student Department of Ophthalmology, Vejle Hospital, Lillebaelt Hospital, Department of Regional Health Research, University of Southern Denmark
1.4	Statistician/data analyst	Lou-Ann C. Andersen, MD, PhD-student Department of Ophthalmology, Vejle Hospital, Lillebaelt Hospital, Department of Regional Health Research, University of Southern Denmark (prepares and conducts analysis) Sören Möller, MSc Maths, PhD, Assoc. Prof Research unit of OPEN - Open Patient data Explorative Network (Odense), Department of Clinical Research, University of Southern Denmark (advisor on analysis preparation)
1.5	Reference to protocol version being used	2A Protocol 116050 Version 6 27.03.25 Approved by the Regional Committees on Health Research Ethics of Southern Denmark
1.6	SAP version and revision history	Version 2, 23-05-25: 4.2 (protocol deviation), 5.1 (An update of the eligibility to reflect the updated criteria in the NISDO study, as the participants are recruited from the randomized clinical non-inferiority NISDO study) and 6.1 (correction for ocular magnification)
1.7	Date for approval of final SAP version	11.06.2025
1.8	Timeframe for conducting the proposed analysis	23.06.2025

Section 2: Introduction

2.1	Describe briefly background, research questions and rationale behind the study	The new Defocus Incorporated Multiple Segments (DIMS) spectacle lens slows the progression of myopia by slowing the longitudinal (axial) growth of the eye in childhood. An increased thickness of the central (subfoveal) choroid is seen after prolonged use, suggesting that the overall eye length (choroidal thickness added to the axial length) is a potential measure of treatment efficacy. However, it is not known whether the use of DIMS lenses has an immediate (within minutes) effect on the choroidal thickness. We will determine the change in subfoveal choroidal thickness within 60 minutes of removal/resumed use of DIMS lenses in children to establish overall eye length as a measure of treatment efficacy.
2.2	Describe briefly objectives and/or hypotheses	*Purpose* To examine temporary changes in the subfoveal choroid thickness after removal and after resumed use of Defocus Incorporated Multiple Segment Spectacle Lenses (DIMS). *Endpoint* 1. Change in subfoveal choroidal thickness at 5 minutes, 10 minutes, 15 minutes, and 30 minutes after removal of DIMS. 2. Change in subfoveal choroidal thickness at 5 minutes, 10 minutes, 15 minutes, and 30 minutes after resuming the use of DIMS. *Hypothesis:* There is a statistically significant decrease in the subfoveal choroidal thickness within 30 minutes of DIMS spectacle lens removal and a statistically significant increase in the subfoveal choroidal thickness within 30 minutes of resumed DIMS spectacle lens use.

Section 3: Study methods

3.1	<u>Study design</u> Describe type of study (i.e. experimental/observational, parallel group/cross over, singlecenter/multicenter ect.) and describe briefly interventions	Prospective experimental singlecenter study.
3.2	<u>Randomization details</u> (if applicable) Describe randomization i.e. allocation ratio, potential factors randomization will be stratified for and describe how and when randomization will be performed	No randomization.
3.3	<u>Sample size</u> Describe calculation of sample size or reference to sample size calculation in study protocol	The number of participants is based on a power calculation for the primary goal with a significance level of 0.05, a power of 80% with a mean change of 6 µm, and an standard deviation (SD) of 7 µm. This gives a total of 13 children. The power calculation is based on results from continues myopic defocus after 30 minutes in the study of the human axial length and choroidal thickness responses to myopic blur condition B, with a mean change in axial length of 6 µm and a

		SD of 7 µm (1). The study's correlation between the change in axial length and choroidal thickness is considered good, thus the change in axial length is used as a pseudo-variable for the expected change in subfoveal choroidal thickness.
3.4	Hypotheses framework Describe hypotheses framework i.e. superiority, equivalence or noninferiority hypothesis testing and which group comparisons will be analysed	H0: - There is a statistically significant decrease in the subfoveal choroidal thickness ≤ 30 minutes of DIMS spectacle lens removal - There is a statistically significant increase in the subfoveal choroidal thickness ≤ 30 minutes of resumed DIMS spectacle lens use. H1: - There is no statistically significant decrease in the subfoveal choroidal thickness ≤ 30 minutes of lens removal - There is no statistically significant increase in the subfoveal choroidal thickness ≤ 30 minutes of resumed lens use meaning that we will test whether there is a statistically significant change in the subfoveal choroidal thickness ≤ 30 minutes of the removal/use of DIMS spectacle lens.
3.5	Statistical interim analyses and stopping guidelines (if applicable) Describe how and when interim analyses will be performed, and potential planned adjustment of significance level due to interim analyses. Describe guidelines for stopping the trial early.	No stopping guidelines. No planned interim analyses.
3.6	Timing of outcome assessments and follow-up Describe time points at which outcomes/covariates will be measured (consider a figure to visualize the time windows of measurements – see appendix)	The study consists of one examination day with SS-OCT (Swept Source Optical Coherence Tomography) measurements on the participant's right eye. The first half of the examination consists of a baseline measurement with Defocus Incorporated Multiple Segment (DIMS) Spectacle Lenses, following 4 measurements within 30 minutes (min) after removing the DIMS spectacle lens (at the 5 min, 10 min, 15 min and 30 min mark). The second half of the examination starts with the resumed use of DIMS spectacle lenses immediately after the 30-minute measurement, following 4 measurements in the remaining 30 minutes (at the 35 min, 40 min 45 min and 60 min mark).
3.7	Timing of final analysis i.e. all outcomes analysed collectively or analyses performed according to planned follow-ups	All outcomes are analysed collectively.

Section 4: Statistical principles and protocol deviations

4.1	Confidence intervals and P-values Specification of level of statistical significance and confidence intervals to be	Level of statistical significance will be 0.05. We will report 95% confidence intervals.

	reported. Describe, if relevant, rationale for adjustment for multiple testing and how type 1 error will be controlled for	
4.2	Adherence/compliance and protocol deviations Define adherence/compliance and how this is assessed in the study. Define protocol deviations and which protocol deviations will be summarized and presented	As there is no direct intervention, possibility for adherence problems is limited. Three main adherence challenges could be expected: - Missing measurements, these will be handled by the mixed effects models, assuming missing at random (MAR) - Measurements at time points different from those specified in the protocol (e.g. 5 minute measurement performed at minute 8). These will be excluded (i.e. replaced by missing) if differences are 5 minutes or more. - Measurements with Image Quality value < 40 or blink artefacts in the foveal region. These will be excluded (i.e. replaced by missing) A technical error in the reading of the autorefractor apparatus output at the time of inclusion in the NISDO study resulted in one child included in the TIDOCT study with a spherical cycloplegic refraction on the measured right eye of –1.75 D, assuming random error. No children were excluded by this error. It was decided to keep the participant in the TIDOCT study.
4.3	Analysis populations Define analysis population i.e. intention-to-treat, per-protocol, complete case, safety population	As no adherence challenges are expected (see 4.2) Intention to treat and per-protocol analyses would be equivalent, and hence only one analysis on all data (as specified in 4.2) will be performed.

Section 5: Study population

5.1	Screening (if applicable) Describe screening data to determine eligibility (i.e. scoring and scales)	This is a study with participants from the DIMS group ≥ 10 years of age. The participants will be recruited from the ongoing study: “Defocus Incorporated Multiple Segment (DIMS) Spectacle Lenses versus Orthokeratology lenses (OKL) for slowing myopia progression in children aged 6-12 years. A non-inferiority randomized clinical trial. The NISDO study” (ClinicalTrials.gov Identifier NCT05134935).
5.1	Eligibility Summarize in- and exclusion criteria	Myopic children ≥ 10 years of age using Defocus Incorporated Multiple Segment Spectacle Lenses (DIMS) from the ongoing study: "Defocus Incorporated Multiple Segment (DIMS) Spectacle Lenses versus Orthokeratology lenses (OKL) for slowing myopia progression in children aged 6-12 years. A non-inferiority randomized clinical trial. The NISDO study" (ClinicalTrials.gov Identifier NCT05134935) Inclusion criteria: -Myopia at the time of inclusion in the NISDO study (inclusive): -2.00 to -4.75 D spherical component and up to -2.50 D of regular astigmatism (both eyes). -Anisometropia at the time of inclusion in the NISDO study ≤ 1.50 D cycloplegic spherical equivalent refractive error. -Best corrected visual acuity (inclusive) at the time of inclusion in the NISDO study: 0.8 Snellen (equivalent to ≥ 3/5 letter on the 0.8 line = 78 ETDRS letters)

		Exclusion criteria: -Manifest or intermittent squint. -Contraindications to the use of OKL comprising (not exhaustive): keratoconus, chronic allergic conjunctivitis, and keratoconjunctivitis sicca. -Previous eye surgery. -Chronic eye disease demanding daily use of eye drops. -Non-compliance to eye examinations (unstable fixation or anxiety towards contact lenses). -Previous myopia control treatment.
5.2	Recruitment and flow chart Specification of steps in the recruitment process i.e. enrolment, screening allocation for use in flow chart (see appendix)	The eligible participants of the NISDO study and their guardian(s) are asked if they want to participate in the study. Their decision does not have any consequences for future treatment or participation in the main study (the NISDO Study). The examination is booked if they want to participate.
5.3	Withdrawal/loss to follow-up Specification on how reason and timing of withdrawal or loss to follow-up will be recorded and presented (i.e. in the flow chart – see appendix)	Will be recorded in the following categories: - Do not wish to participate.
5.4	Baseline patient characteristics List of baseline characteristics and how these data will be descriptively summarized in a “Table 1” (see appendix)	Normal distribution: Mean, SD Not at normal distribution: Median, Range (As determined by a normal quantile-quantile plot) - Sex (male vs. female, fraction or percent) - Age at enrolment (years, mean and SD) - Time since handout of the first pair of DIMS (months, median and range) - DIMS Spectacle spherical equivalent refractive error (SER, median, range) -Daily non-wear (hours, median, range): Patient reported mean number of waking hours pr. day without spectacles. Descriptive data on the performed measurements will be reported similarly in a separate table: - Time from baseline measurement (+DIMS) to the following measurements (-DIMS: 5 minutes, 10 minutes, 15 minutes, 30 minutes. +DIMS: 35 minutes, 40 minutes, 45 minutes, 60 minutes) (minutes, mean and SD) - Time of day from first to last measurement (hh:min start & end, median, range). - Measurement quality assessment: number of re-segmented scans (N, mean and SD)

Section 6: Analysis

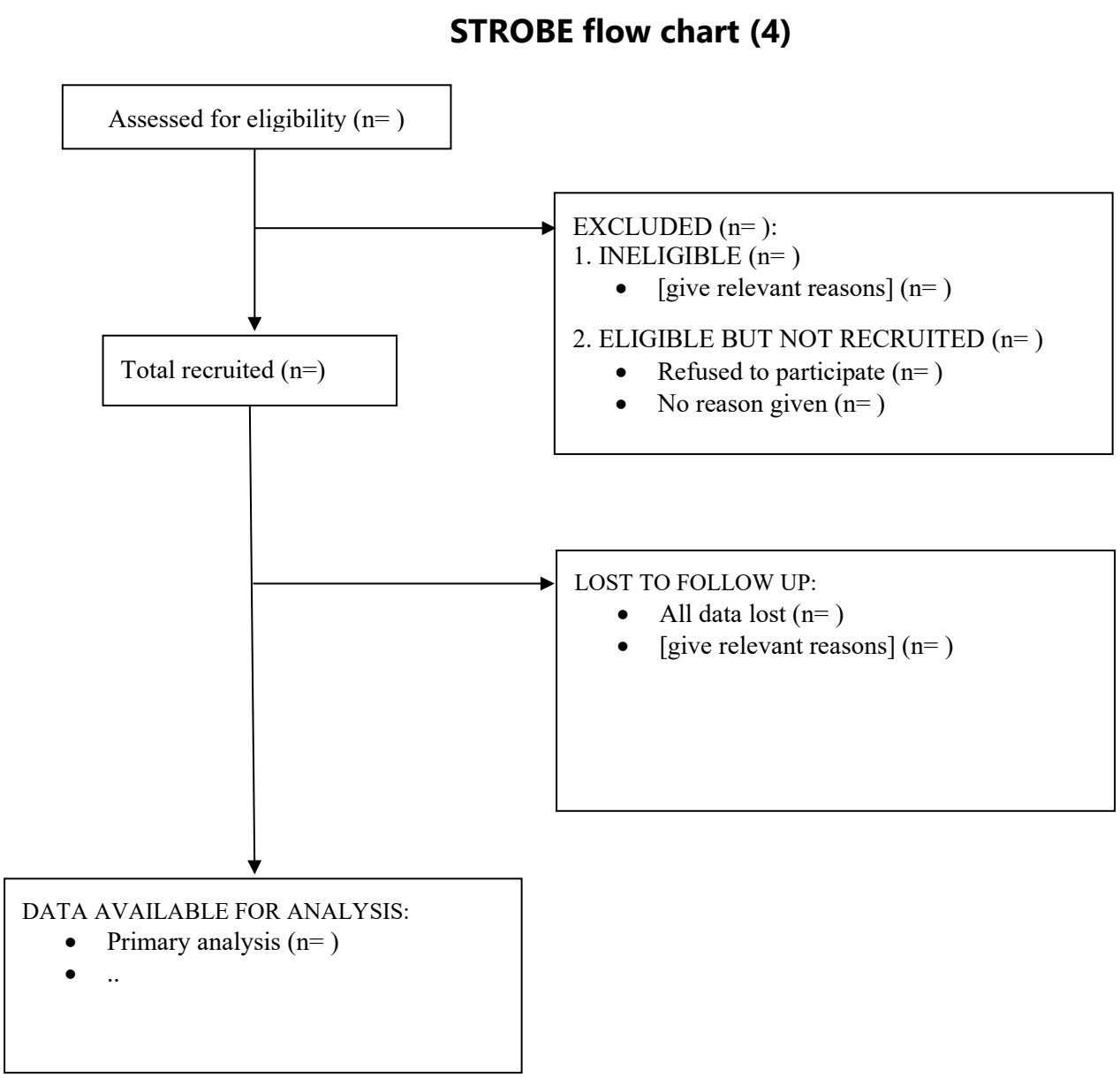
6.1	Exposure and outcome definitions Describe details on exposure i.e. assessment, definitions, units and thresholds or the intervention/treatment under study. List and describe details on primary and secondary outcomes i.e. definition of outcome and timing, specific clinical measurements and units (i.e. mmol/mol) or any calculation or transformation of data to derive the outcome (i.e. sum score, change from baseline, logarithm, quality-of-life scoring algorithm)	Primary Outcome Measure: Subfoveal Choroidal thickness. Thickness is measured in um [Time Frame: During 60 minutes] The choroidal thickness is corrected for ocular magnification using the axial length, mean corneal radius, and refractive error entered in an algorithm.
6.2	Primary analysis methods Describe in details which statistical methods will be used (i.e. regression), how treatment effects will be presented (i.e. which effect measure - OR, HR etc.) and if estimates will be adjusted for covariates (see appendix). If analyses will be adjusted for covariates, describe how the sufficient adjustment set will be defined (i.e. using DAGs) Describe methods used to check assumptions (i.e. normality, proportional hazards) behind the statistical models, and alternative methods if assumptions about distribution do not hold.	We will make a linear mixed effects regression model with the participant as random intercept using subfoveal choroidal thickness as the dependent variable and time point as the (categorical) independent variable, with baseline as reference time point. Moreover, the analyses will be repeated with 30 minutes as the reference time point, for endpoint 2. Regression models will not be adjusted for any covariates, as the hypotheses of interest compare each child with themselves, and the sample size limits possibilities for adjustments. We will report the resulting coefficients (mean changes in subfoveal choroidal thickness) with standard errors (SE) and 95% confidence intervals. Model assumptions will be checked using normal quantile-quantile plots for both residuals and random effects.
6.3	Additional analysis methods Describe any planned sensitivity and subgroup analysis including how subgroups will be defined (see appendix).	No additional analyses will be performed.
6.4	Missing data Describe how missing data will be explored and which assumptions and methods will be used to handle missing data (i.e. multiple imputation)	Missing data are assumed to be missing at random (MAR) and will be handled by the mixed effects regression model.

6.5	Harms (only applicable in experimental studies) Describe the collection of safety data i.e. data on severity, expectedness, causality. Describe grouping and analyses planned i.e. incidence analyses on grade 3-4 events only.	The study is safe with no risks and no or minimal discomfort to the participant. The DIMS spectacle lens treatment is considered safe as there are no theoretical or published side effects.
6.6	Statistical software Specify statistical packages to be used for the analyses	Stata

1. Delshad S, Collins MJ, Read SA, Vincent SJ. The human axial length and choroidal thickness responses to continuous and alternating episodes of myopic and hyperopic blur. Tsai DC, editor. PLOS ONE. 2020 Dec 2;15(12):e0243076.

Appendix: Figure and table templates

5.2-3 Modified low chart template for observational studies



5.4 Baseline table ("Table 1" & "Table 2") template

Table 1: Characteristics of the study population

	All (N = xx)
Age at enrolment, year	
Mean (SD) (or median, range)	xx (xx)
Sex, N (%M)	xx (xx%)
Time since DIMS spectacle lens Handout, months	
Mean (SD) (or median, range)	xx (xx)
SER spec, D	
Median (Range)	xx (xx-xx)
Daily non-wear, hours	
Median (Range)	xx (xx-xx)

DIMS = Defocus Incorporated Multiple Segment; Daily non-wear = Patient reported mean number of waking hours pr. day without DIMS spectacles lenses; N = number; SD = standard deviation; M = Male; SER spec = spectacle spherical equivalent refractive error; D = dioptre.

5.4 Descriptive table ("Table 2") template

Table 2: Descriptive data on the performed measurements

	All (N = xx)
Time from baseline measurement to the following measurements, mm:ss	
05 min - Mean (SD) (or median, range)	xx (xx)
10 min - Mean (SD) (or median, range)	xx (xx)
15 min - Mean (SD) (or median, range)	xx (xx)
30 min - Mean (SD) (or median, range)	xx (xx)
35 min - Mean (SD) (or median, range)	xx (xx)
40 min - Mean (SD) (or median, range)	xx (xx)
45 min - Mean (SD) (or median, range)	xx (xx)
60 min - Mean (SD) (or median, range)	xx (xx)
Time of day of first and last measurement, hh:mm	
First measurement - Mean (SD) (or median, range)	xx (xx)
Last measurement - Mean (SD) (or median, range)	xx (xx)
Quality assessment: number of re-segmented SS-OCT scans	
Mean (SD) (or median, range)	xx (xx)

mm:ss: = minuets:seconds; hh:mm = hours:minutes, N = number; SD = standard deviation; SS-OCT = Swept Source Optical Coherence Tomography.

6.2 Primary Analysis ("Table 3") template

Table 3: Linear mixed effects regression model with the participant as random intercept using subfoveal choroidal thickness as the dependent variable and time point as the (categorical) independent variable, with baseline as reference time point. Moreover, the analysis is repeated with 30 minutes as the reference time point.

	Mean subfoveal choroidal thickness um (SD)	Difference from baseline um (95% CI)	Difference from 30 minutes um (95% CI)
Baseline	xx (xx)	(Reference)	
05 minutes	xx (xx)	xx (95% CI xx-xx)	
10 minutes	xx (xx)	xx (95% CI xx-xx)	
15 minutes	xx (xx)	xx (95% CI xx-xx)	
30 minutes	xx (xx)	xx (95% CI xx-xx)	(Reference)
35 minutes	xx (xx)	xx (95% CI xx-xx)	xx (95% CI xx-xx)
40 minutes	xx (xx)	xx (95% CI xx-xx)	xx (95% CI xx-xx)
45 minutes	xx (xx)	xx (95% CI xx-xx)	xx (95% CI xx-xx)
60 minutes	xx (xx)	xx (95% CI xx-xx)	xx (95% CI xx-xx)

um = micrometre; SD = standard deviation

References for further reading on SAP and reporting guidelines

1) Gamble C, Krishan A, Stocken D, Lewis S, Juszczak E, Dore C, et al. Guidelines for the Content of Statistical Analysis Plans in Clinical Trials. JAMA. 2017;318(23):2337-43. doi: 10.1001/jama.2017.18556.

2) Chan AW, Tetzlaff JM, Altman DG et al. SPIRIT 2013 statement: defining standard protocol items for clinical trials. Ann Intern Med. 2013 Feb 5;158(3):200-7. doi: 10.7326/0003-4819-158-3-201302050-00583.

3) Schulz KF, Altman DG, Moher D. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. BMJ. 2010;340:c332.

4) Vandenbroucke JP, von Elm E, Altman DG, Gotzsche PC, Mulrow CD, Pocock SJ, et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. Int J Surg. 2014;12(12):1500-24. doi: 10.016/j.ijsu.2014.07.014.

5) Yuan I, Topjian AA, Kurth CD, et al. Guide to the statistical analysis plan. Pediatr Anesth. 2019;29:237–242. https://doi.org/10.1111/pan.13576