

## EXTENDED REPORT

## Lower limits of fluorescein and indocyanine green dye for digital cSLO fluorescence angiography

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**Background:** With the advent of digital confocal scanning laser ophthalmoscopy it is possible to detect low levels of fluorescence. Here we used a novel confocal scanning laser ophthalmoscope (cSLO) to determine lower limits of dye required for fluorescein (FL) and indocyanine green (ICG) angiography.

**Methods:** A cSLO (Heidelberg retina angiograph 2, Heidelberg Engineering, Dossenheim, Germany) with an optically pumped solid state laser (488 nm) for FL and a diode laser (790 nm) for ICG angiography (FL/ICG-A) was used. 62 FL-As were performed in 53 patients and 45 ICG-As were performed in 39 patients with neovascular age related macular degeneration. The volume and overall dye content of bolus injections was gradually tapered (FL: 500 mg, 250 mg, 200 mg, 166 mg, 100 mg; ICG: 25 mg, 20 mg, 15 mg, 10 mg, 5 mg, 2.5 mg), while dye concentrations were kept constant at 100 mg/ml for FL and at 5 mg/ml for ICG. Images were obtained 1, 5, 15, and 30 minutes after dye injection. Image quality was evaluated by two independent readers using standardised criteria.

**Results:** For amounts down to 166 mg for FL and to 5 mg for ICG, sufficient image quality was achieved during all phases following injection. Only late phase images showed less contrast compared to typically used dye amounts, which was irrelevant for interpretation and clinical management.

**Conclusions:** With the increased sensitivity of this novel cSLO system, amounts of injected dye during FL-A can be reduced to one third for FL and to one fifth for ICG without relevant loss of image quality or information compared to conventionally used dye levels. These amounts can be used for routine angiography and allow relevant savings for units performing FL-A.

Fluorescence angiography (FL-A) with fluorescein (FL) and indocyanine green (ICG) represents an important diagnostic tool for evaluation of a wide spectrum of retinal diseases.<sup>1–10</sup> Two different imaging systems have been introduced for FL-A. Conventional fundus cameras were initially used by Nowotny and Alvis.<sup>11</sup> As a result of the pioneering work of Webb and co-workers, scanning laser ophthalmoscopes (SLOs) have become available for routine application.<sup>12–14</sup> Fundus camera based systems use a bank of capacitors that are discharged through a xenon flash tube for FL-A. Photographs are recorded on film or digitised via a CCD (charge coupled device) camera on a computer system. Frame rate is limited with these systems for various reasons, including time required for recharging the capacitors. With the advent of cSLO and recent further technological improvements, it is now possible to detect very low levels of fluorescence in the eye.<sup>15–17</sup> Compared to the flash light used to illuminate the fundus of conventional fundus cameras,<sup>18</sup> cSLOs use a laser beam with adequate wavelength for excitation of physiological or diagnostically injected fluorophores. In confocal systems, light from a confocal plane is detected while light from planes anterior and posterior to the plane of interest are suppressed. Although optical properties of the human eye still limit image resolution, cSLO angiography allows for high contrast images with a high horizontal resolution of the posterior pole of the eye. Furthermore, it is possible to record images in real time at a high frame rate and therefore to visualise detailed dynamic processes. Because fluorescein and ICG have different spectral characteristics, simultaneous angiographies can be performed using SLO systems.<sup>19–23</sup>

Given the high sensitivity of cSLO systems compared to conventional camera systems, we hypothesised that lower

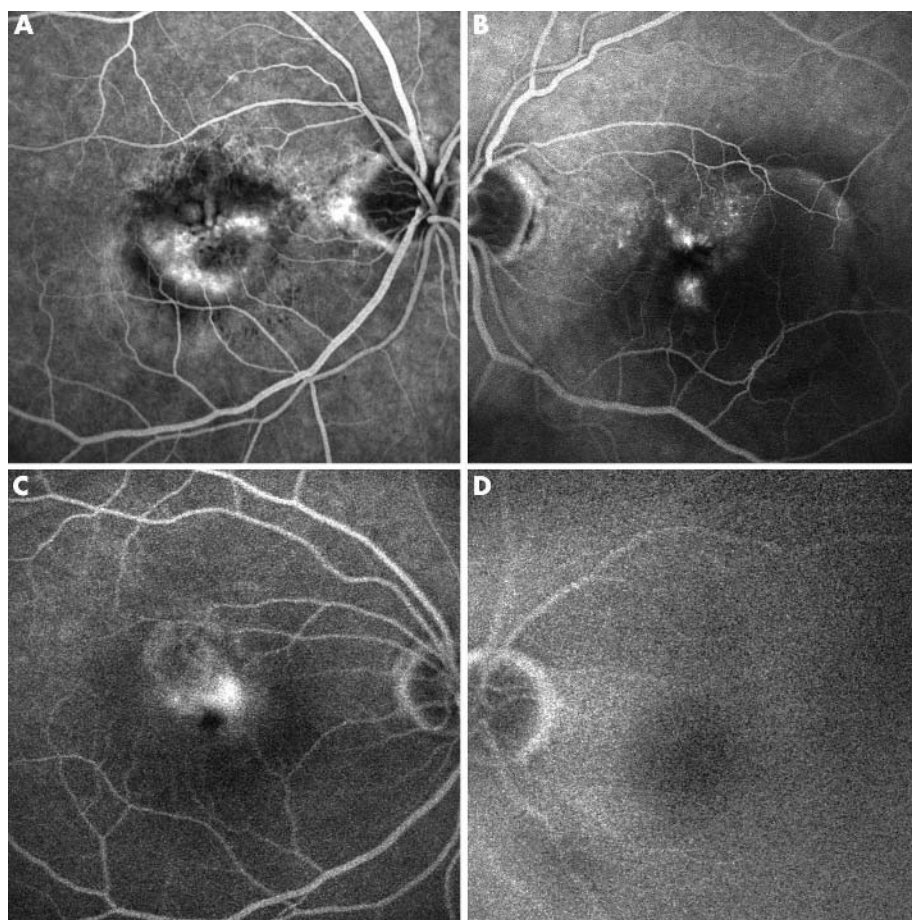
amounts of FL and ICG dye would be required for routine FL-A. Here we determined the lower limit of dye using stepwise reduction in FL or ICG injected for FL-A using a novel cSLO.

## PATIENTS AND METHODS

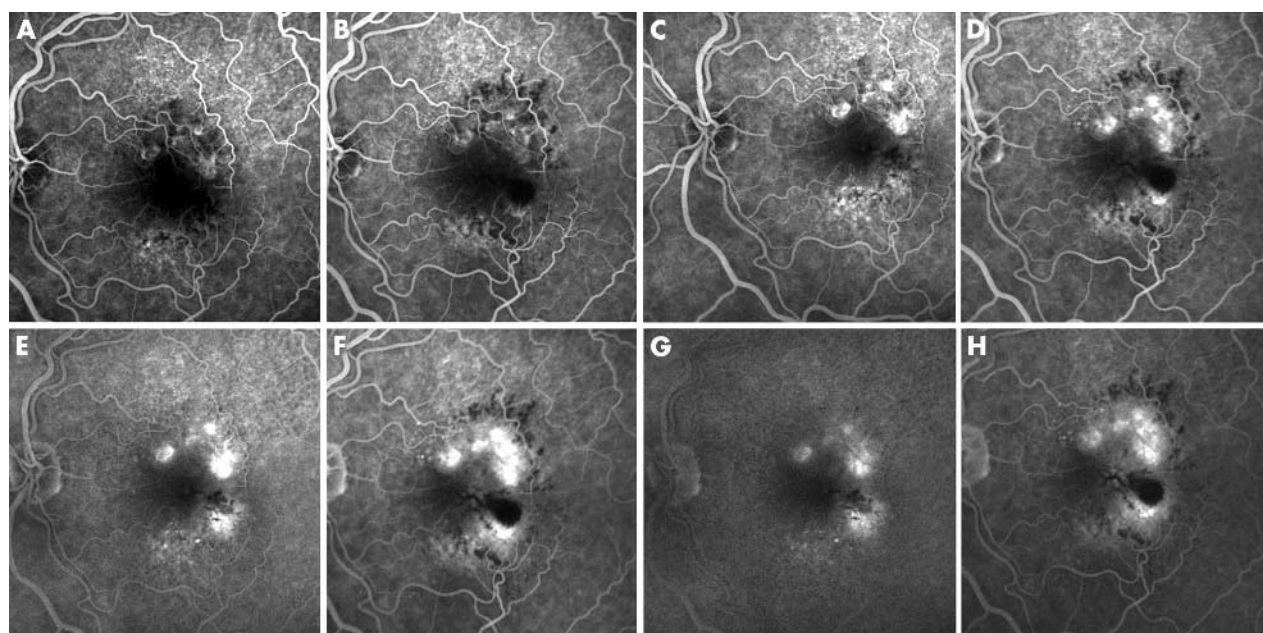
For consecutive FL angiography (A) and ICG-A, a novel cSLO (HRA2, Heidelberg Engineering, Dossenheim, Germany) was used. The principle of cSLO for FL-A has been described previously.<sup>15 21 23–25</sup> The HRA2 uses an optically pumped solid state laser (488 nm) for FL-A and a diode laser (790 nm) for ICG-A. Maximum retinal irradiance is approximately 2.0 mW/cm<sup>2</sup> and therefore lies below the limits established by the American National Standards Institute and other international standards.<sup>26</sup> Emission is recorded between 500 nm and 700 nm with a detection efficiency of 85% for FL-A images, and above 810 nm with a detection efficiency of 66% for ICG recordings. A digital zoom at an angle of 30° was used to obtain digital images of 768×768 pixels using the continuous or single image acquisition mode at a line scan frequency of 8 kHz (maximum 16 frames per second). For digital image processing, the included software was used (Heidelberg Eye Explorer, HEE, Heidelberg Engineering, Dossenheim, Germany).

We performed 62 FL-As on 53 patients (20 male, 33 female; age 76.5 (SD 7.5) years), and 45 ICG-As on 39 patients (15 male, 24 female, age 76.1 (9.0) years) seen in the retina outpatient clinic of the department of ophthalmology, University of Bonn. The patients all had neovascular age

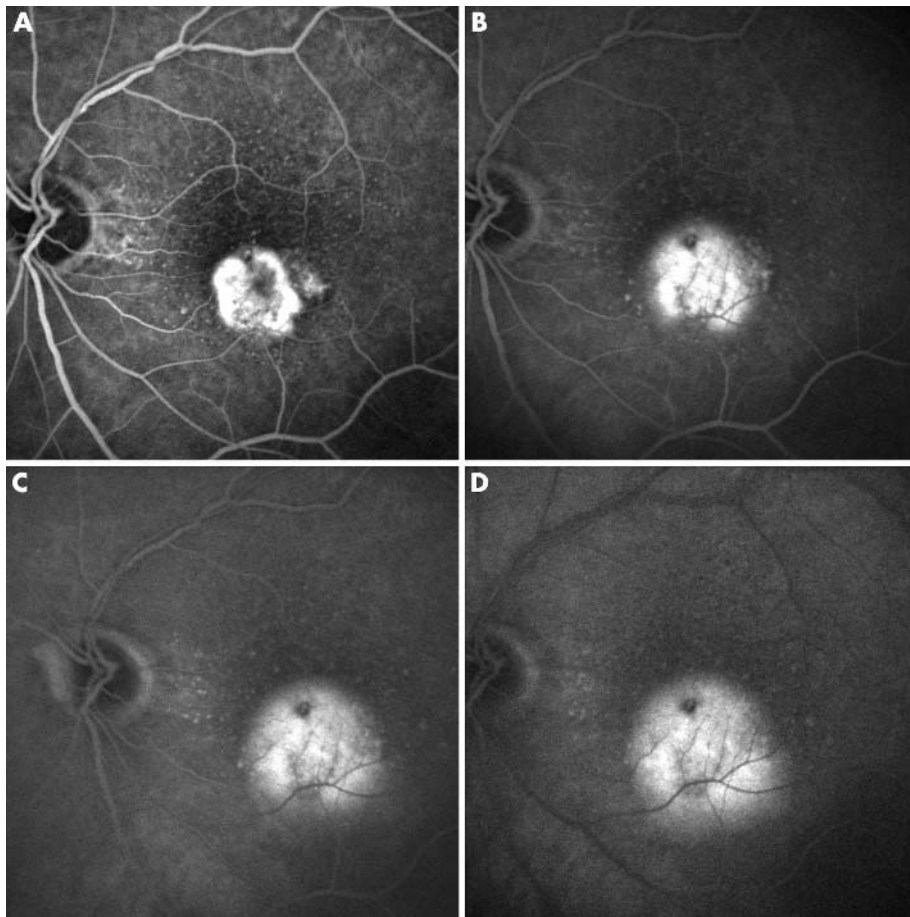
**Abbreviations:** CCD, charge coupled device; cSLO, confocal scanning laser ophthalmoscopy; FA, fluorescence angiography; FL, fluorescein; FL-A, fluorescein angiography; ICG-A, indocyanine green angiography; ICG, indocyanine green



**Figure 1** Grading system with four categories for fluorescence angiographies. (A) "Very good" (5 minutes), (B) "good" (5 minutes), (C) "poor image quality" (15 minutes), (D) "not readable" (15 minutes).



**Figure 2** Fluorescein angiography of a 79 year old pseudophakic patient with neovascular age related macular degeneration. This figure shows results from injection of 500 mg (B, D, F, H) and 166 mg (A, C, E, G) of fluorescein dye. Note progression of disease after 6 weeks (top row) with more leakage and haemorrhage, which is independent from the amount of administered fluorescein. (A, B) 1 minute, (C, D) 5 minutes, (E, F) 15 minutes, (G, H) 30 minutes.



**Figure 3** Fluorescein angiography of a 82 year old phakic patient using 166 mg fluorescein. All details of the minimally classic choroidal neovascularisation can be identified during all phases of angiography. (A) 1 minute, (B) 5 minutes, (C) 15 minutes, (D) 30 minutes.

related macular degeneration (AMD). FL-As and ICG-As were performed consecutively. The volumes and dye amounts (mg) of the bolus injections were gradually tapered for both FL (500 mg, 250 mg, 200 mg, 166 mg, 100 mg; fluorescein 10%, Alcon Pharma GmbH, Freiburg, Germany) and ICG (25 mg, 20 mg, 15 mg, 10 mg, 5 mg, 2.5 mg; ICG-Pulsion, Pulsion Medical Systems AG, Munich, Germany), while dye concentrations were maintained at 100 mg/ml for FL and at 5 mg/ml for ICG. Patients were randomly assigned to different volumes and dye amounts. All injections were performed by the same injecting physician in an attempt to achieve similar injection dynamics for all angiograms. In each patient, 30° images were recorded at 1, 5, 15, and 30 minutes after dye injection.

Inclusion criteria included media clear enough to allow satisfactory imaging, especially absence of advanced lens opacities, and informed written consent. Patients with contraindications for FL or ICG injection (for example, allergies to shellfish, penicillin, or iodine; pregnancy; known allergies to either FL or ICG; or insufficient compliance and

nystagmus) were excluded.<sup>27</sup> The study was reviewed by the appropriate ethics committee and performed in accordance with the ethical standards laid down in the Declaration of Helsinki.<sup>28</sup>

Quality of images was evaluated by two independent readers using standardised criteria and classified as “very good,” “good,” “poor image quality,” or “not readable” (fig 1). In case of a discrepancy, a third reader was asked to arbitrate. The readers were not aware of the dye amounts injected.

Statistical analyses were performed using commercially available software (SPSS, SPSS GmbH Software, Munich, Germany). Results at each time point of examination (1, 5, 15, and 30 minutes) were tested for interaction of the amount of dye and image quality as quantitative variables using  $\chi^2$  and linear by linear association tests.

**RESULTS**

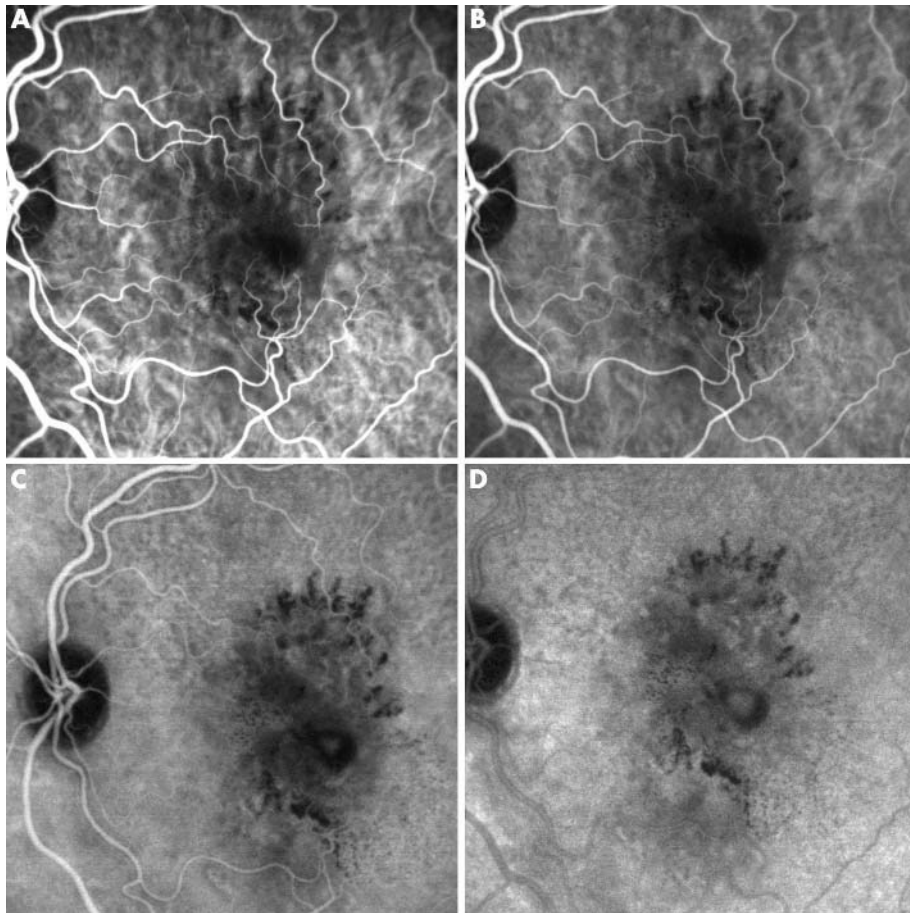
We performed 62 FL-As in 53 patients, using an identical concentration of dye in five different bolus volumes (table 1). While 30 patients were phakic, 23 patients had undergone cataract surgery. Four images of each FL-A (at 1, 5, 15, and 30 minutes after injection) were classified into the categories as mentioned above by two independent readers. The two readers graded a total of 428 FL-A and ICG-A images. While complete agreement was achieved in 68.7% of the cases, in 30.8% there was a minor difference in grading—that is, within one step of the grading scale. During the earlier phases of FL-A (1 minute and 5 minutes after injection), a total of 96.7% of images after 1 minute and 85.5% of images after 5 minutes following injection were classified either as “very good” or “good,” and none was classified as “not readable”

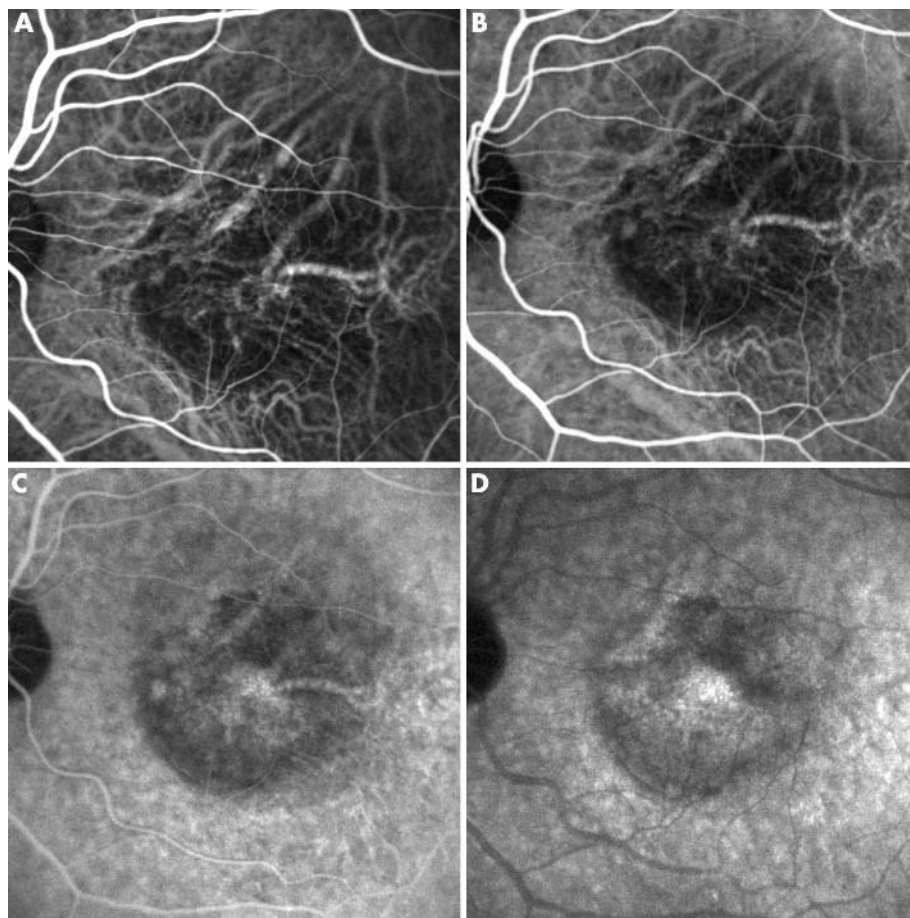
**Table 1** Fluorescein angiographies and dye amounts used

No (n=62)	FL amount	FL injected volume (100 mg/ml)
11	100 mg	1.00 ml
17	166 mg	1.66 ml
10	200 mg	2.00 ml
9	250 mg	2.50 ml
15	500 mg	5.00 ml

**Table 2** Results of the evaluation of fluorescein angiographies

FL	Very good	Good	Poor	Not readable	No
<b>1 minute</b>					
100 mg	6 (54.5%)	5 (45.5%)	0 (0%)	0 (0%)	11
166 mg	9 (52.9%)	7 (41.2%)	1 (5.9%)	0 (0%)	17
200 mg	8 (80%)	2 (20%)	0(0%)	0 (0%)	10
250 mg	7 (77.8%)	1 (11.1%)	1 (11.1%)	0 (0%)	9
500 mg	11 (73.3%)	4(26.7%)	0 (0%)	0 (0%)	15
	41 (66.1%)	19 (30.6%)	2 (3.2%)	0 (0%)	62
<b>5 minutes</b>					
100 mg	3 (27.3%)	6 (54.5%)	2 (18.3%)	0 (0%)	11
166 mg	8 (47.1%)	5 (29.4%)	4 (23.5%)	0 (0%)	17
200 mg	7 (70%)	3 (30%)	0 (0%)	0 (0%)	10
250 mg	7 (77.8%)	0 (0%)	2 (22.2%)	0 (0%)	9
500 mg	10 (66.7%)	4 (26.7)	1 (6.7%)	0 (0%)	15
	35 (56.5%)	18 (29%)	9 (14.5%)	0 (0%)	62
<b>15 minutes</b>					
100 mg	0 (0%)	8 (72.7%)	3 (27.3%)	0 (0%)	11
166 mg	3 (17.6%)	8 (47.1%)	5 (29.4%)	1 (5.9%)	17
200 mg	0 (0%)	10 (100%)	0 (0%)	0 (0%)	10
250 mg	2 (22.2%)	5 (55.6%)	2 (22.2%)	0 (0%)	9
500 mg	8 (53.3%)	5 (33.3%)	2 (13.3%)	0 (0%)	15
	13 (21%)	36 (58.1%)	12 (19.4%)	1 (1.6%)	62
<b>30 minutes</b>					
100 mg	0 (0%)	7 (63.6%)	3 (27.3%)	1 (9.1%)	11
166 mg	1 (5.9%)	8 (47.1%)	6 (35.3%)	2 (11.8%)	17
200 mg	0 (0%)	7 (70%)	1 (10%)	2 (20%)	10
250 mg	0 (0%)	7 (77.8%)	0 (0%)	2 (22.2%)	9
500 mg	7 (46.7%)	6 (40%)	2 (13.3%)	0 (0%)	15
	8 (12.9%)	35 (56.5%)	12 (19.4%)	7 (11.3%)	62

**Figure 4** Indocyanine green (ICG) angiography of a 79 year old pseudophakic patient using the full amount of 25 mg of dye. (A) 1 minute, (B) 5 minutes, (C) 15 minutes, (D) 30 minutes.



**Figure 5** Indocyanine green (ICG) angiography of a 73 year old phakic patient using 5 mg of dye. Only the late phase images show somewhat less contrast. (A) 1 minute, (B) 5 minutes, (C) 15 minutes, (D) 30 minutes.

(table 2). Statistical analysis did not reveal a significant difference between different amounts of FL during the early phases (linear by linear association = 0.291 for 1 minute and 0.114 for 5 minutes) (table 2). At 15 minutes after FL bolus injection, only one image (166 mg FL) was classified as “not readable.” For all volumes and dye amounts 21% of FL-A images were classified as “very good” and 58.1% as “good”; higher amounts of FL led to an overall better classification (linear by linear association = 0.004). At 30 minutes after FL bolus injection, no image obtained with 500 mg fluorescein was classified as “not readable.” The rates for “not readable” late phase fluorescein angiograms are given in table 2. Statistical analysis indicated overall better image quality using higher amounts of FL (linear by linear association = 0.002) (table 2) (figs 2 and 3).

For the ICG dye, we performed 45 ICG-As in 39 patients, using identical dye concentrations in different volumes

(table 3). Of the group, 19 patients were phakic and 20 patients were pseudophakic. Four frames of each ICG-A (1, 5, 15, and 30 minutes after injection) were evaluated. A proportion of 71.1% of images taken 1 minute after injection were rated as “very good” (table 4). At 5 minutes after injection, ICG-A with 2.5 mg and 5 mg of fluorescence dye led to single images with “poor” image quality; in general, images were significantly better with higher amounts of dye (linear by linear association = 0.09 for 1 minute and 0.05 for 5 minutes) (table 5). The late phase images overall had slightly worse ratings with lower levels of dye (linear by linear association = 0.00 for 15 minutes and 0.00 for 30 minutes) (table 5). Out of all 15 minute frames, only one image (20%) taken with 2.5 mg and two images (15.3%) taken with 5 mg dye were classified as “not readable.” Proportions for 30 minutes shots classified as “not readable” were 60% (three out of five) in the 2.5 mg group and 30.8% (four out of 13) in the 5 mg group. At higher amounts, none of the images was classified as “not readable” (table 4) (figs 1 and 4).

**Table 3** Indocyanine green angiographies and dye amounts used

No (n = 45)	ICG amount	ICG injected volume (5 mg/ml)
5	2.5 mg	0.50 ml
13	5 mg	1.00 ml
8	10 mg	2.00 ml
8	15 mg	3.00 ml
5	20 mg	4.00 ml
6	25 mg	5.00 ml

**DISCUSSION**

With the advent of cSLO, low levels of fluorescence in the human eye can be recorded. Recommendations regarding the amount of dye to be injected for FL-A (500 mg) and ICG-A (25 mg) are based on conventional camera systems that were introduced decades ago. With a stepwise reduction of dye volumes injected while maintaining the same concentrations, the current study indicates that for routine clinical purposes, 166 mg of fluorescein and 5 mg of ICG are sufficient when using a new cSLO system.

The use of only one third of the FL-A and one fifth of the ICG-A conventionally used dye amounts, respectively, allows

**Table 4** Results of the evaluation of indocyanine green (ICG) angiographies

ICG	Very good	Good	Poor	Not readable	Total
<b>1 minute</b>					
2.5 mg	2 (40.0%)	3 (60.0%)	0 (0%)	0 (0%)	5
5 mg	7 (53.8%)	6 (46.2%)	0 (0%)	0 (0%)	13
10 mg	5 (62.5%)	3 (37.5%)	0 (0%)	0 (0%)	8
15 mg	8 (100%)	0 (0%)	0 (0%)	0 (0%)	8
20 mg	5 (100%)	0 (0%)	0 (0%)	0 (0%)	5
25 mg	5 (83.3%)	1 (16.7%)	0 (0%)	0 (0%)	6
	32 (71.1%)	13 (28.9%)	0 (0%)	0 (0%)	45
<b>5 minutes</b>					
2.5 mg	1 (20%)	3 (60%)	1 (20%)	0 (0%)	5
5 mg	4 (30.8%)	8 (61.5%)	1 (7.7%)	0 (0%)	13
10 mg	4 (50.0%)	4 (50%)	0 (0%)	0 (0%)	8
15 mg	6 (75%)	2 (25%)	0 (0%)	0 (0%)	8
20 mg	3 (60%)	2 (40%)	0 (0%)	0 (0%)	5
25 mg	5 (83.3%)	1 (16.7%)	0 (0%)	0 (0%)	6
	23 (51.1%)	20 (44.4%)	2 (4.4%)	0 (0%)	45
<b>15 minutes</b>					
2.5 mg	0 (0%)	1 (20%)	3 (60%)	1 (20%)	5
5 mg	0 (0%)	6 (46.2%)	5 (38.5%)	2 (15.4%)	13
10 mg	1 (12.5%)	4 (50%)	3 (37.5%)	0 (0%)	8
15 mg	1 (12.5%)	5 (62.5%)	2 (25%)	0 (0%)	8
20 mg	4 (80%)	1 (20%)	0 (0%)	0 (0%)	5
25 mg	3 (50%)	3 (50%)	0 (0%)	0 (0%)	6
	9 (20%)	20 (44.4%)	13 (28.9%)	3 (6.7%)	45
<b>30 minutes</b>					
2.5 mg	0 (0%)	0 (0%)	2 (40%)	3 (60%)	5
5 mg	0 (0%)	3 (23.1)	6 (46.2%)	4 (30.8%)	13
10 mg	1 (12.5%)	5 (62.5%)	2 (25%)	0 (0%)	8
15 mg	2 (25%)	5 (62.5%)	1 (12.5%)	0 (0%)	8
20 mg	4 (80%)	1 (20%)	0 (0%)	0 (0%)	5
25 mg	3 (50%)	1 (16.7%)	2 (33.3)	0 (0%)	6
	10 (22.2%)	15 (33.3%)	13 (28.9%)	7 (15.6%)	45

for relevant savings in times of cost constraints for all health systems. These savings would especially apply to high volume medical retina departments. Furthermore, there is evidence to suggest that side effects of fluorescein, such as nausea, are dose dependent,<sup>27</sup> and it would be expected that using lower dye amounts would reduce the incidence of these side effects. Therefore, it appears prudent to use minimally necessary dye amounts for fluorescence angiography.

The analyses of both FL-A and ICG-A images indicated that image quality diminishes in late phases, especially with a reduction in dye amounts, which was the expected outcome. Lower amounts of dye below the threshold mentioned above would not be sufficient to obtain enough information from the angiographic examinations. Minor reductions in contrast and resolution appear irrelevant for the clinical management of patients in a routine setting. For example, as long as the borders of a classic choroidal neovascularisation (CNV) are clearly delineated in the early phase and leakage of dye is identified in later frames, the physician can determine his or her therapeutic strategy (for example, photodynamic therapy). In addition, other factors may be more important for

interpreting angiographic findings than optimal image resolution.<sup>29</sup>

For special purposes—for example, identification of minuscule structures visualised during angiography such as flow in the capillary perifoveal network or recordings for illustrations, the use of conventional dye amounts may be considered to achieve optimal resolution. Again, such use does not appear to be necessary for routine angiographies.

Lower dye amounts can also be used for simultaneous FL-A and ICG-A using the cSLO system. We have shown previously that both FL and ICG dye can be mixed in one syringe and injected as bolus with subsequent simultaneous recordings.<sup>17–30</sup>

Various limitations have to be considered when interpreting this study. We only investigated patients with various manifestations of AMD; however, we assume that these findings would be comparable in the presence of other retinal pathologies. Furthermore, only patients with relatively clear media were examined. Advanced lens opacities may impair fluorescence image quality by absorption both in the excitation and absorption spectra of the fluorescent dyes. Therefore, lower amounts of dye may be disadvantageous in eyes with advanced cataract, and it may be prudent to use standard amounts under such circumstances. The relatively small number of subjects in each subgroup represents a limitation of the study and needs to be considered when interpreting the data. However, since there was overall relatively little variability within the subgroups, we would assume that larger numbers would in essence not yield other results. Finally, the classification of image quality is obviously a subjective evaluation; however, there is no objective means available to accomplish more accurate ratings.

In summary, this new cSLO (HRA2) allows for detection of low levels of fluorescence. We have shown that it is possible to use amounts of fluorescein and/or ICG dyes for routine

**Table 5** Statistical analysis of ICG-A for evaluation of the relation between dye amount and image quality using different amounts of dye

Time (minutes)	Linear by linear association (<0.05 significant)
1	0.09
5	0.05
15	0.00
30	0.00

fluorescence angiography that are lower than those previously used for conventional camera based systems. This finding also allows for relevant savings in expenses.

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Competing interests: none declared

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