Hoya		Schott		Corning		Omega		Chroma	
UV 30	-	*WG320*	hh	*0-52*	hh	*330cflp* (310)	2	*E340lp*	h
UV 32	-	*WG335*	-	*3-75*	-	*392cflp* (380)	1	*E350lp* (330)	3
UV 34	-	*WG345*	h	*3-74*	-	*410cflp* (395)	1	*E370lp* (360)	3
L1B	-	*WG360* (350)	1	*3-73*	-	*427aglp* (410)	1	*E380lp* (370)	3
UV 36	hh	*GG375*	-	*3-72*	hh	*450cflp* (440)	1	*E390lp* (370)	3
L37 (355)	1	*GG385* (360)	1^+	*3-71* (475)	1	*450ablp* (445)	1	*E400lp* (395-540)	2
L39 (365)	1	*GG395* (365)	1^+	*3-70* (490)	2	*468aglp* (460)	1^+	*E410lp* (415-540)	3
L38 (370)	2	*GG400*	-	*3-69* (505)	1	*493aglp* (480)	1	*E420lp*	hh
L40	-	*GG420*	hh	*3-68* (530)	1^+	*500cflp* (495)	1	*E430lp*	hh
L42 (420)	1	*GG435*	hh	*3-67* (540)	1	*511aglp* (500)	1	*E435lp* (435)	2
YA44	hh	*GG455* (445)	2	*3-66*	-	*530aglp* (520)	1	*E450lp* (450-540)	2
YA46	-	*GG475*	hh			*550aglp* (540)	1	*E460lp* (460)	2
YA48	hh	*GG495*	h					*E470lp* (470-660)	1
YA50	hh	*OG515*	h					*E480lp* (480-680)	1
Y52	h	*OG530*	3					*E490lp* (485-690)	3
O54	h	*OG550* (535)	2					*E495lp*	hh
O56	h	*OG570* (555)	1					*E500lp*	h
								E510lp (505-720)	1
								E520lp	h
								E530lp (530-720)	2

Table III

Longpass filter evaluation: The performance of filters available from 5 sources is listed. Filters with "-" in the rating column were not available at the time of investigation. Filter names that contain "lp" are dielectric filters while all others are color glass filters. Rating "1^+" is lowest autofluorescence and "hh" highest. Auto fluorescence was measured with excitation spectra at 350, 450 and 550 nm emission wavelength. Autofluorescence intensity was rated at excitation wavelengths between the longpass cut off wavelength and 50 nm below. Rating "1^+" was equivalent with $< 1 * 10^5$ calibrated counts on the detector, rating "1" < 5, rating "2" < 20, rating "3" < 50, "hh" > $200 * 10^5$ counts. Small numbers next to the filter name indicate the lowest wavelength with 1% transmission. Some dielectric filters had a limited blocking range which is indicated. Shaded boxes represent the filters used for the system.

The filter cups were loaded with carefully selected longpass filters. For minimal system autofluorescence longpass filters need to be placed in a collimated beam path and at distance from a system aperture. However, such a configuration was limited by mechanical constraints from combining the fiberoptic adapter and the filterwheel. To minimize autofluorescence produced by these filters, all standard longpass filters available from five different manufacturers were ordered and their optical characteristics measured. The five manufacturers were Chroma Technology Crop., Omega Optical Inc. (Brattleboro, VT) and Newport Industrial Glass (Stanton, CA) representing color glass filters from Hoya, Schott and Corning. The blocking range was determined as well as the characteristic transmission fluctuations of long pass filters created with dielectric coatings. The autofluorescence excitation spectra were measured at 350, 450 and 550 nm emission wavelength on a SPEX Fluorolog II fluorimeter by placing each filter into the sample chamber and collecting fluorescence in a front face configuration. Filters were rated between 1 and 3 where 1 represented low fluorescence. Additionally high and very high autofluorescence was noted.

The system analysis revealed several possible sources of systematic errors. Stray light which could originate in both the light source and the optical analyzer was determined to be insignificant. External background signals can be accurately removed from each measurement if every measurement is repeated under the same conditions but with the light source shutter closed. Autosignals were a major concern. In general they originate from fluorescing components in the optical path. Determining autofluorescence requires as a scattering reflector which does not fluoresce itself. An unpolished quartz surface resembled tissue reflectance the closest and had minimal autofluorescence (Table 2). The optical components with largest potential of creating autofluorescence were identified as the longpass filters suppressing the excitation light in the optical analyzer. The evaluation of the longpass filters revealed that only a few filters are suitable for our device (Table 3). Most filters chosen were based on dielectric coatings and manufactured especially for low autofluorescence. Since the fluorescence efficiency of tissue in the blue-green excitation range is low, good autofluorescence suppression is essential.

However this is also a range where only a few filters were optimal. When measuring tissue, for excitation wavelengths above 400 nm, low autofluorescence at 550 nm is important while for excitation wavelengths below 400 nm, low autofluorescence at 450 nm is important. Low autofluorescence at 350 nm is important below 320 nm excitation. The selected longpass filters are listed in Table 3 and were 330cflp (Omega), e350lp (Chroma), gg385 (Schott), 392cflp (Omega), L42 (Hoya), 450cflp (Omega), 468aglp (Omega), 493aglp (Omega), 3-69 (Corning), 3-68 (Corning) and 550aglp (Omega).