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Towards non-invasive Wilson's disease progression monitoring based on corneal copper content

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Abstract: Patients suffering from Wilson's disease accumulate copper in their body. Copper load may become visible as Kayser-Fleischer (KF) rings - colorful copper-sulfur granules in the base of the cornea. Reoccurrence of KF rings in patients under anti-copper medication may be a sign for ineffective treatment. This work proposes a hand-held sensing system based on infrared reflectivity measurement of the cornea as a potential method for regular at-home progression monitoring. The intensity of the LEDs used is safe for operation on the human eye. As proof of concept, reflectivity was measured when placing the sensor onto samples of white paper that had copper tape of different sizes centered within the sensitive area. Further, reflectivity was determined for different solutions of copper sulfate in saline that were poured into adapter bowels fitting the sensor head, with Cu^{2+} concentrations of the samples deducted from literature. While the prototype system can be used to differentiate between two copper strips of 6 and 12 mm^2 or larger, a different measuring principle would be needed to also determine the concentration of copper sulfate solutions. Since these two *in-vitro* models cannot fully represent the complex *in-vivo* molecular conditions of patients' corneas, additional measurements on patients' eyes are necessary to evaluate the potential of the proposed sensing system.

Keywords: Wilson's disease, Kayser-Fleischer ring, copper, cornea, non-invasive, reflectivity

1 Introduction

Wilson's disease (WD) is a hereditary recessive disorder (prevalence 1 : 30,000) characterized by impaired excretion of ingested copper that is caused by dysfunctional hepatic enzymes. Increased Cu^{2+} levels in liver or blood as well as high urinary (instead of biliary) copper excretion indicate WD [1]. To limit irreversible cell damage from accumulated copper, early and adequate medication in addition to a low-copper diet is necessary [2]. Free copper may also lead to neurological symptoms seen in 45 % of the patients, often accompanied by a colorful accumulation of copper in the cornea of the eye known as Kayser-Fleischer (KF) ring [1, 2].

Figure 1: Hand-held prototype sensing system using infrared LEDs and a photo diode.

1.1 Kayser-Fleischer rings

KF rings appear as an arc or ring along the perimeter of the iris, their color usually ranging from brown to yellow / golden or green [2, 3]. They do not cause any discomfort in the patient and only in more severe cases, they are visible to the naked eye [2]. Examination using a slit lamp is a well-established method to search for KF rings, however success depends on the experience of the ophthalmologist since KF rings may hardly be seen in patients with dark eyes or in an early stage of the disease.

In WD patients, increased levels of copper ions can be measured throughout the entire cornea, also in the central region. Corneal copper is therefore not limited to the peripheral KF rings, However it is only in the iridocorneal angle of the eye that Cu^{2+} is linked to sulfur-containing molecules forming granular complexes that are stored within the Descemet's membrane (the basement membrane adjoining the corneal endothelium) [11, 12]. These granules, which are between 5 to 350 nm [13], cause the colorful appearance of KF rings.

It is not clear which parameter determines the extent to which KF rings develop. A significant correlation between cupriuresis and copper deposition in the superior cornea was

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Table 1: Review of KF ring detection methods

Method	Description	Limitations
Slit lamp examination	Gold standard, success depends on the experience of the physician [3] and on the color contrast between iris and KF staining	Low sensitivity [3]
Gonioscopy	Contact-based examination of the underlying iridocorneal angle where KF rings are most dominant	Difficult with neurological symptoms, e.g. tremor [3, 4]
Confocal microscopy	Contact-based, KF rings seen as hyper-reflective granules, significant correlation between deposits and patient cupriuresis.	Requires good training [5, 6]
OCT	KF rings seen as hyper-reflective band	Expensive diagnostic tool, in ophthalmic practices only [3]
Image processing	Determining the stained area of the cornea, deciding whether the pattern found is indicative of a KF ring. No specific equipment needed.	Sensitivity may depend on the color contrast between KF ring and iris [7, 8]
Topography and densiometry by Scheimpflug imaging ("Pentacam")	KF rings seen as hyper-reflective band, optical density of the posterior peripheral cornea may be increased in WD patients with and without KF rings	In ophthalmic practices only [9]
X-ray excitation spectrometry	Beam is directed through the cornea sideways, signal depends on copper content	Safety precautions needed, risk of damage to radiation-sensitive children's eyes [10]

found in [5]. Having reviewed several studies, Chevalier et al. conclude that KF rings are expected to fade or disappear after successful long-term treatment, however their disappearance does not directly correlate with the reduction of other clinical symptoms or blood levels. Still, the reappearance of faded KF rings may indicate ineffectiveness of the current treatment [3].

1.2 Diagnostic approaches

While general WD symptoms such as hepatitis may appear in many other diseases as well, the presence of KF rings is a distinctive and therefore valuable diagnostic sign seen only in few conditions besides WD [3, 14]. To facilitate the diagnostic process, examination of the eyes to find KF rings should be a reliable and easily available procedure. As of today, different methods were described to detect them. Some allow for quantitative determination of the extent of the rings, which would be necessary to use KF rings also to non-invasively monitor the impact of the current WD medication and the progression of the disease. A summary is given in table 1.

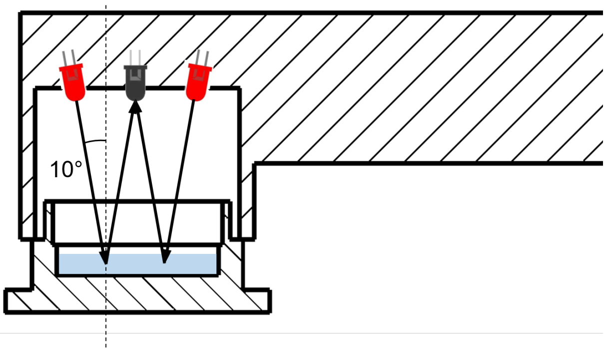
1.3 Reflective measurement

Even if there is no general correlation between KF rings and severity of WD, tracking the changes in KF ring development may be of interest for the individual patient. Monitoring may serve either as an additional, non-invasive means to confirm recovery after start of treatment or as an early warning if the condition is worsening. If a non-invasive system for use at their regular doctor's office or at home was available, it might increase patient comfort and reduce the need to consult different specialists, which is a financial burden in some countries.

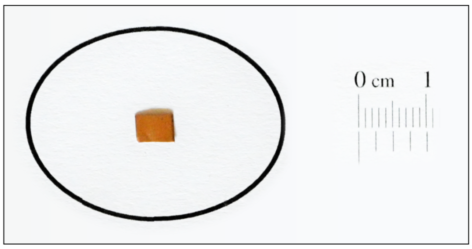
Previous work has shown that the copper-rich layer appears hyper-reflective in infrared (IR) imaging systems [3, 4, 9]. As opposed to methods relying on images in the visible spectrum, in reflectivity measurement the color of the iris should only play a minor role, therefore allowing usage by the vast group of dark-eyed people worldwide. In this paper, a compact and inexpensive setup is used to measure IR reflection of in vitro models. Rather than recording absolute copper levels, the proposed system is intended for tracking the relative individual progression of corneal reflectivity only. This may be sufficient for the intended purpose of progression monitoring.

2 Materials and Methods

For potential use at home, a prototype hand-held device was designed. The housing printed from PLA includes an eyepiece for repeatable positioning as well as an Arduino Nano microcontroller (Arduino S.r.l., Monza, IT) controlling two infrared LEDs (850 nm). A SFH 213 photo diode (OSRAM, Premstaetten, AT; maximum sensitivity at 850 nm) is placed between the LEDs. The diode current depending on the intensity of reflected light is converted to a 0-5 V voltage signal through a trans-impedance amplifier circuit and read by the micro controller. Through a display and a push button, the user can start the measurement and read the result. Figure 1 shows the prototype. A measurement comprises collection of the diode intensity signal for 2 s and subsequent calculation of the mean as output signal.



(a)



(b)

Figure 2: Measurement setups. a) Sectional view of prototype on vessel filled with copper solution. The IR light emitted by the LEDs at an angle of 10° is reflected inside the solution and captured by the photo diode. b) Copper tape as used for functionality test.

2.1 Safety

To confirm that operation of the device is safe to the eyes, the maximum irradiance (power per area) and radiance (power per area and solid angle) in front of the eye are calculated from the specifications of the LEDs. Maximum irradiance not harmful for the cornea is $10\,\mathrm{kW/m^2}$, maximum radiance tolerated by the retina is $420\,\mathrm{kW/(m^2 \cdot sr)}$ [15].

2.2 In vitro models of copper deposits

For proof of concept of the sensing system, two different in vitro models were used mimicking the copper-loaded cornea. For higher copper levels, higher reflectivity of the infrared light should be seen.

2.2.1 Copper tape

As a basic test of functionality, pieces of copper tape were cut to different sizes ($6\,\mathrm{mm^2}$ to $90\,\mathrm{mm^2}$) and reflectivity was recorded. Each sample was centered on a white area matching the shape of the eyepiece as seen in figure 2b. Per sample, 20 measurements were recorded as described above, then the overall mean and standard deviation were calculated.

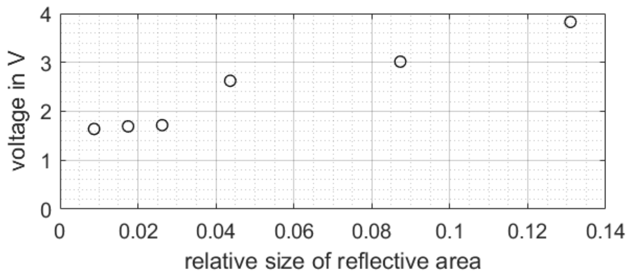


Figure 3: Results of copper tape reflectivty measurement. The x-axis shows the relative size of the copper samples in relation to the elliptical sensitive area seen in figure 2b.

2.2.2 Copper sulfate solution

Copper ions in the cornea of a WD patient are most numerous in the Descemet's membrane where larger sulfur-containing granules form [12]. To model the copper load, different amounts of copper sulfate pentahydrate ($CuSO_4 \cdot 5\,H_2O$) were dissolved in saline solution. Typical concentrations were derived from the work of Johnson and Campbell [12] as well as Belkin et al. [10] who measured copper concentration of the entire cornea of healthy and WD patients.

2 ml of each solution were poured into a black PLA vessel corresponding to the shape of the sensing device, which was placed on top as seen in figure 2a. Absorption characteristics at 850 nm are not known for the PLA filament used, therefore reflectivity of the dry and empty vessel was recorded before measuring the samples. During each measurement, 1000 subsequent data points were recorded. Beside six different copper solutions, also pure saline solution and the empty vessel were measured. All concentrations are given in table 2.

3 Results

3.1 Safety

The maximum irradiance emitted by the device was calculated as $1.8\,\mathrm{kW/m^2}$. Maximum radiance resulted in $26.3\,\mathrm{kW/(m^2 \cdot sr)}$. Both values are well below the limits stated in section 2.1, therefore regular usage with the human eye may be considered safe.

3.2 In vitro models

With copper tape samples, reflectivity increases with sample area. The difference in reflectivity is significant for all mea-

Table 2: Measurement results of copper sulfate samples. No. 5 to 7 were used to access sensor sensitivity.

No.	Sample	Cu^{2+} concentration	Mean $\pm$ standard deviation in V
E	empty vessel	-	0.20 ± 0.01
0	pure 0.9 % NaCl solution	-	0.74 ± 0.03
1	healthy	0.9 ppm	0.64 ± 0.03
2	WD, treated	4 ppm	0.73 ± 0.11
3	WD, Cu^{2+} elevated (10x healthy level)	10 ppm	1.14 ± 0.15
4	WD, Cu^{2+} strongly elevated (60x healthy level)	60 ppm	0.36 ± 0.03
5	Cu^{2+} 600x healthy level	600 ppm	0.87 ± 0.01
6	Cu^{2+} 6,000x increased	0.6 %	0.23 ± 0.01
7	Cu^{2+} 60,000x increased	6 %	0.80 ± 0.03

sured samples ($p < 0.01$ from Student's t-test). Signal amplitudes are shown in figure 3.

For the copper solutions, the data given in table 2 show no correlation between Cu^{2+} concentration and reflectivity.

4 Discussion

This paper describes a new method of corneal copper detection for WD patients. The presented prototype is based on an optical principle using light intensities that are unlikely to harm the eye and was proven to be able to distinguish different percentages of reflective area. From corneal copper concentrations given in literature, a simple *in-vitro* model for the copper-loaded cornea was derived, based on Cu^{2+} dissolved in saline solution. However, it was not possible to differentiate between different concentrations with the proposed setup.

The models used were relatively simple with copper ions dissolved in water and do not fully represent the optical properties of the copper particles linked to sulfuric proteins in the cornea where copper ions are aggregated. Therefore the proposed copper sulfate solution may be an inadequate model: Even if granule size is described as 5 to 350 nm [13], the actual corneal deposits must be larger aggregations of granules. Otherwise they would not reflect infrared light (> 800 nm). However, IR illumination was successfully used to display hyperreflective corneal layers in confocal microscopy and OCT (see table 1). To evaluate suitability of the sensing system, a follow-up study should repeat the measurements on WD patients with and without KF rings. In case of negative results, other novel methods of non-invasive molecule-specific diagnostics may be worth adapting, e.g. multi-spectral optoacoustic tomography.

It is not yet clear whether continuous monitoring of corneal copper levels is a suitable means of progression monitoring for WD patients. As an inexpensive measurement principle that could be directly made accessible to the patients, it is however an approach worth pursuing.

Author Statement

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