A METHOD FOR THIARUBRINE CANALS EXTRACTION IN OPTICAL COHERENCE TOMOGRAPHY IMAGES OF SCHKUHRIA PINNATA ROOTS

JOANNA SEKULSKA-NALEWAJKO 1  JAROSŁAW GOCŁAWSKI 1  MARZENA WIELANEK 2  EWA GAJEWSKA 2  
SZYMON TAMBORSKI 3

1 Lodz University of Technology, Institute of Applied Computer Science, Stefanowskiego 18/22, PL 90-924 Lodz, Poland, {jgoclaw, jsekulska}@kis.p.lodz.pl, 2 University of Lodz, Department of Plant Physiology and Biochemistry, Banacha 12/16, PL 90-237 Lodz, Poland, {mawie, ewagaj}@uni.lodz.pl, 3 Nicolaus Copernicus University, Institute of Physics, Faculty of Physics, Astronomy and Informatics, Grudziądzka 5, PL 87-100 Toruń, Poland, {sodalis}@fizyka.umk.pl

Abstract. This paper presents a method of automatic recognition of thiarubrine canals in images obtained with Optical Coherence Tomography technique. The plant material was the Ri-transformed root culture of South American herb Schkuhria pinnata. The series of high-resolution OCT B-scans for the study were collected using custom made experimental system operating light of 800 nm central wavelength. The method reduces significant artefacts and uses region growing approach adapted to specific features of OCT images. Results of the identification have been compared with data obtained by specialist for selected B-scans. The algorithm accuracy was also verified using a simple numeric phantom.

1 Introduction

Optical Coherence Tomography (OCT) is one of the most advanced, noninvasive optical imaging technologies reconstructing the spatial structure of tested materials by coherence gating of near-infrared light scattered back on the internal layers of the object. As an light-interference-based technique it shows high sensitivity and superb precision. The use of light of spectral region around 800 nm assures preservation of high sensitivity up to 3 mm penetration in semi-transparent media which makes it an excellent tool for 3D imaging of biomedical objects. It enables in situ imaging with a resolution approaching that of histology (up to 1.5 µm), but without the need for tissue dissection or staining. The unique features of OCT would enable a broad range of research, which might not only complement many of the imaging technologies available today, but also will potentially reveal previously unseen morphological, dynamic, and functional changes in dif-
ferent model systems and materials.

This technology was initially used mainly in medicine. Over past years there have been increasing applications of 3D-OCT for imaging of biological micro-structures such as human retina [8], human skin [22], blood vessels [9] and models in developmental biology [7]. The rapid advancements in the field paved the way to functional applications such as measurement of flow in retina blood vessels [21] or analysis of the dynamics of the imaged objects, such as humans lens during accommodation [11]. These techniques are largely based on the analysis on series of images acquired in well defined time intervals. Recently it can also be observed increased interest in using this technique in the plant sciences and agriculture. Based on the results of recent studies it can be seen, that by using OCT it is possible to observe the distribution of internal morphological changes in plant leaves or seeds [15, 17]. This causes the possibility of application of this technique in screening where it is necessary to fast diagnosis of the plants and the introduction of protective treatments. For example it has been shown that changes within the plant tissues due to viral, bacterial or fungal infection thanks to OCT method can be detected much sooner than in the case of observation by means of conventional detection techniques (visible symptoms can only be seen after at least 2-3 weeks after infection or 4-5 if the latency period is long) [5, 6]. This has an impact on the acceleration of decision protection against the disease with suitable control program, such as fungicide spraying. Other advantages of the use of OCT in the study of plant material is to eliminate the time-consuming procedures or techniques that destroying tissue structures. Moreover, the non-destructive character of the OCT allows using one and the same object for observation over a long period of time.

In this study we apply 2D and 3D scanning OCT technology to imaging of plant roots in order to recognize the subsurface structure and detect regions of the potential accumulation of metabolites. The plant material was the Ri-transformed hairy root culture of South American herb Schkuhria pinnata. We verify if the use of OCT method enables the study of spatial distribution of thiarubrine canals over the entire length of the vital roots and to develop an image processing method of canals extraction. Usefulness of those methods for visualization of efficiency of techniques used for intensification of thiarubrines biosynthesis will be investigated by biologists in further experiments.

2 Biological material

Ri-transformed root cultures are considered promising sources for the biotechnological production of bioactive metabolites [12, 13]. They usually reflect the chemistry of the maternal plant therefore would be an interesting system to examine the biosynthesis of bioactive compounds as well as to their large scale production. South American herb Schkuhria pinnata synthesizes several bioactive compounds, including thiarubrines and thiophenes. Thiarubrines are a group of 1,2-dithia-3,5-cyclohexadiene (1,2-dithiin) polyacetylene derivatives characterized by having an intense red color and photo-labile structure, and have been found to possess both light-mediated and light-independent antibacterial, antifungal, antiviral, insecticidal and anticancer properties. Exposure of thiarubrines to light results in their rapid decomposition to thiophenes and change their cytotoxicity. Because of possible photo-auto-toxicity the thiarubrines are contained within resin canals in the cortex and periderm of roots (as well as stems and leaves), which run longitudinally along the plant (as illustrated on the example of Ambrosia chamissonis steam, Fig. 1). In photosynthetic tissues canals additionally are enclosed by a photoprotective layer of anthocyanin pigments that absorbs visible light and thereby prevents thiarubrine photodegradation [18].
Heterotrophic and fotomixotrophic hairy roots of *S. pinnata* were established after transformation with wild strain A4 of *Agrobacterium rhizogenes*. For microscopic determination of the presence and localization of the red thiarubrines transformed roots were cross-sectioned [1]. Sectioning using resin techniques was not successful due to pigment degradation under the conditions of fixing and embedding. Hand-sectioning gave much better results and revealed that roots compartmentalized thiarubrines in discernible pockets between the double endodermal layers. Because formation of canals are very important for the maintenance of the photolabile compound, culture conditions, as well as procedures for intensification of metabolite biosynthesis must be adapted to accommodate this.

### 3 OCT experimental setup and imaging protocol

The series of B-scans for the study were collected with the use of Spectral OCT microscopic device developed it the Institute of Physics at Nicolaus Copernicus University (Torun, Poland). Because of the great sensitivity of thiarubrines to light the plants were stored in the absence of light till the moment of scanning. The single roots were scanned submerged in the water.

The OCT setup consisted of the fibre-based Michelson interferometer combined with the high-performance spectrometer. The sample arm of the interferometer was terminated with Olympus LUCPLFLN 20/0.45 objective which provided lateral resolution at the level of 2 µm. The imaging beam was scanned over the sample with the use of pair of galvo scanners (Cambridge Technology, UK). The two scanners of perpendicular axis enabled precise realization of the raster scan. The laser light (800 nm central wavelength, full width at half maximum of the spectrum: 150 nm) was delivered from the output of titanium-sapphire femtosecond laser (Femtolasers, Austria) and assured the in-depth resolution of ca. 2µm. The power of probing light was 2 mW. The acquisition of the light in the spectrometer was performed with the use of CMOS line scan camera (Basler Sprint spL4096-140km, Germany). The center 2048 pixels out of the total number of 4096 of the pixel line of the camera were used. The sensitivity of the system was 97 dB. The camera interface was Camera Link which enabled acquisition with the maximum speed of 140 kHz (140 000 image lines per second). The dynamic range of the camera was 12 bit.

The post processing of the raw Spectral OCT data was performed according to the established procedures [2] which are based on the Fourier transformation and resulted in bitmaps files which were the target objects of analysis.
4 Image preprocessing - artefact reduction

The series of B-scans analysed in the paper included artefacts, which existence can significantly influence the detection results of the root canals [4]. The distinctive, example artefacts are presented in Fig. 2(a). They appear in two different forms:

- as thin, bright horizontal lines passing through the air above the surface of root sample,
- as the groups of vertical lines fully or fragmentary bright (distorted A-scans).

The artefacts of the last form typically disappear after several B-scans. They cross the sample tissue and therefore hinder the detection of root tissue regions. Fortunately they can be easily identified in the upper parts of slice images, which correspond to an air layer above the root sample. The common limit of "air" image regions can be set-up for all scans interactively or explicitly as some percent of the image height. The artefacts are isolated in these regions and reduced independently for single image slices. The reduction is performed as illustrated in Fig. 3.

The sums of intensities across the rows of upper image part given by Eqn. (1) provide the vertical projection of intensities (Fig. 3) with regional maximum values corresponding to the artefact positions.

\[
\forall z, \quad S_z(x) = \sum_{y=0}^{y_{max}} I_z(x,y),
\]
Fig. 3: The illustration of a vertical projection function $S_z(x)$ for the upper part of a B-scan. $H_z(x)$ - the binary function of artefact bars around local maxima of $S_z(x)$, $T$ - the artefact significance threshold

where $I_z(x, y)$ denotes B-scan of the image slice $z$, $x \in [0, X)$, $y \in [0, y_{\text{max}}]$, $z \in [0, Z)$, $y_{\text{max}} = 0.4 \times Y$. Only sufficiently high local maxima of $S_z(x) > 0.5 \times \max_x (S_z(x))$ are considered as artefact bars with $H_z(x) = 1$ in Eqn. (2).

$$\forall z, H_z(x) = \begin{cases} 1 & S_z(x) > T \\ 0 & \text{otherwise} \end{cases},$$

(2)

where the significance threshold $T = 0.5 \times \max_x (S_z(x))$. In each row $z$ image pixel intensities on both sides of the fixed artefact bar are symmetrically extended to the bar distance $[x_i, x_j]$ according to Eqn. (3).

$$I_z(x_i + \xi, y) = I_z(x_i - \xi, y),$$
 $$I_z(x_j - \eta, y) = I_z(x_j + \eta, y),$$

(3)

where $\xi = 0, \ldots, d_i$, $\eta = 0, \ldots, d_j$, $d_i = \lfloor (x_j - x_i)/2 \rfloor$, $d_j = d - d_i$. Image boundary effects and the bundles of closely located lines are not discussed here.

The left and right half of the bar are replaced by the left and right-side pixel intensities respectively.

The horizontal artefacts in the “air” zone are eliminated in a similar way, after exchanging the roles of rows and columns in an image slice.

5 Thiarubrine canal identification algorithm

Identification of *Schkuhria pinnata* thiarubrine root canals is the problem of retrieval quantitative data from OCT images, which solution must take account of the presence of strong artefacts. Some of them as degraded image scans were initially reduced in a way proposed in Section 4. The cut edge or mirror artefacts can be a result of software errors, while other are object or operator dependent (motion artifact, off-center artifact, degraded image scan, mirror artifact). Additionally all images include a lot of speckle noise [4, 14]. To detect canal region similarly to a human operator the proposed method should ignore rare, strongly white pixels or less bright subregions. Such a method robust to less significant pixel intensity changes can recognize only approximately fuzzy boundaries of tissue material. If the extraction concerns a single image region it is preferred to process its space only instead of all image pixels. Such an approach reduces both memory and time requirements during method’s execution. Following the above considerations a simple heuristic method of region segmentation has been proposed.

The algorithm of this method consists of the steps listed below:

1. mark the set of pixels $p_i(x, y, z)$ located in the region of low infra-red reflection,
2. mark the seed pixel $p_S(x, y, z)$ inside of the region,
3. evaluate the region intensity threshold $T$ as:

$$T = \text{mean}(I(p_i)) + k \times \text{stdev}(I(p_i)),$$

(4)

where $k \in [0.85, 1]$, $\text{mean}(\cdot)$ and $\text{stdev}(\cdot)$ functions denote respectively the mean and standard deviation values of pixel intensities [20],
4. extract the region specified by the seed pixel $p_S$ according to the Algorithm 1.
The proposed segmentation algorithm uses well-known region growing idea [10, 19], which needs the seed pixel inside of the region to start the growing process. The verification of pixel intensity condition given in Eqn. (4) requires both the knowledge of mean grey-value and its standard deviation inside of the region. The pixel grey-level (intensity) information is obtained interactively from freehand drawing inside of the region in any image slice (Fig. 4). At the growing process only the six nearest space neighbours of a current pixel are taken into account. The main feature of the proposed approach is the extension of the region growth condition \( I(p) < T \) from testing the intensity of a current pixel \( p \) to testing its all 6-connected neighbours \( q \) inside of the ball mask \( M(p, R) \) as shown in the Algorithm 1, in line 11. It is assumed that at least half of the ball neighbour pixels, located in the image cube, should fulfil the growth condition. This approach implicitly assumes that possible narrow region protrusions of the width comparable with the ball diameter are of interest, because they cannot be preserved after segmentation. The same feature of the algorithm prevents the leakage of segmentation result outside of the actual region, because of partially vanishing tissue boundaries.

**Algorithm 1** The algorithm for root chamber region identification

**Input:** \( I \) – root image, \( p_S \) – seed pixel, \( T \) – intensity threshold, \( M(p, R) \) – neighbour ball mask around a pixel \( p \)

**Output:** \( I_L \) – binary image with extracted region

\[ \textcircled{1} I_L, I_C \leftarrow \{0\}, \{Q_i\} \leftarrow \text{init} \]
\[ \textcircled{2} p \leftarrow p_S \]
\[ \textcircled{3} n \leftarrow 0 \]
\[ \textcircled{4} \textbf{repeat} \]
\[ \textcircled{5} \quad \textbf{foreach} q \in N_6(p) \land I_C(q) = 0 \textbf{ do} \]
\[ \textcircled{6} \\ \quad I_C(q) \leftarrow 1 \]
\[ \textcircled{7} \\ \quad \text{push} q \text{ in } \text{rand}\{Q_i\} \]
\[ \textcircled{8} \\ \textbf{end foreach} \]
\[ \textcircled{9} \quad \textbf{while} \neg \text{empty any}(Q_i) \textbf{ do} \]
\[ \textcircled{10} \\ \quad \text{pop} p \text{ from } \text{rand}\{Q_i\} \]
\[ \textcircled{11} \\ \quad S \equiv \{I(q) < T : q \in M(p, R) \land q \in I\} \]
\[ \textcircled{12} \\ \quad \text{if med}(S) = 1 \textbf{ then} \]
\[ \textcircled{13} \\ \quad I_L(p) \leftarrow 1 \]
\[ \textcircled{14} \\ \quad n \leftarrow n + 1 \]
\[ \textcircled{15} \\ \textbf{end if} \]
\[ \textcircled{16} \textbf{end while} \]
\[ \textcircled{17} \textbf{until} \text{empty all}(Q_i) \lor n \geq \text{size}(I) \]

The algorithm uses a set of queues randomly chosen to store the neighbours \( N_6(p) \) of a processed pixel \( p \). The neighbour pixels are then popped from the queues to test their participation in the region of interest. At the image boundaries only the pixels \( q \) common to the ball mask \( M \) and image cube \( I \) are taken into account.

### 6 Experimental results

The experiments of testing the proposed method were carried out on a PC with Intel(R) Core(TM) i3-2120, 3.30 GHz CPU and 16 GB GDDR3 RAM memory. The algorithm was executed in MATLAB 2013b environment.
on 64-bit operating system Windows 7 Professional. It has been developed both in the form of M scripts and C++ MEX files.

The *Schkuhria pinnata* root tissue was scanned in a cube of $1250 \times 1450 \times 1350$ $\mu$m in $X$, $Y$ and $Z$ directions respectively. The acquired 3D images of the size $600 \times 2048 \times 600$ px were stored as a series of $600 \times 2048$ bitmap files assembled in separate folders. The tasks of reading and collecting bitmaps into a three-dimensional image in memory and the identification of thiarubrine canal region have been written as C++ procedures and compiled with Visual Studio 2008 compiler. The task of degraded line correction described in Section 4 has been designed as M-function. The accuracy of the proposed identification method was tested using well-known $F_1$-score and Jaccard $J$ coefficients [16]. They compare the pixel set $R_A$ of an automatically extracted canal region with its reference $R_B$ according to Eqn. (5) and Eqn. (6).

$$F_1 = \frac{2 |R_A \cap R_B|}{|R_A| + |R_B|},$$

$$J = \frac{|R_A \cap R_B|}{|R_A \cup R_B|},$$

where $|\cdot|$ denotes the cardinality of an appropriate pixel set. The reference region sets were obtained in two ways:

1. by manual labelling of the root canal region in two dimensions,

2. by the creation of phantom objects of known size and shape, similar to the region of interest in shape and pixel intensity.

The manual method was performed by experts in Corel Photo Paint XV environment with the use of color masks, pencil and eraser [3]. Because this approach is very difficult to implement it has only been applied to 4 selected slices of the examined region in a selected image.

The phantom method applies an elongated ellipsoid object (Fig. 6):

$$E(a, b, c) \subset I[X \times Y \times Z],$$

$$a = 0.4 \times X, \quad b = 0.08 \times Y, \quad c = 0.2 \times Z,$$

where the semi-axis $a$ is positioned in $X$ direction and the image size components are $X = Y = Z = 512$ px. The ellipsoid exterior is filled with the rectangular pattern of root tissue randomly selected from an original OCT image slice. The interior is prepared similarly from rectangle samples of canal region intensity.

Tab. 1: The list of identification accuracy indices evaluated for the selected region slices labelled by experts

<table>
<thead>
<tr>
<th>slice ID</th>
<th>$F_1$-score</th>
<th>Jaccard</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.953</td>
<td>0.932</td>
</tr>
<tr>
<td>2</td>
<td>0.922</td>
<td>0.906</td>
</tr>
<tr>
<td>3</td>
<td>0.905</td>
<td>0.883</td>
</tr>
<tr>
<td>4</td>
<td>0.895</td>
<td>0.868</td>
</tr>
</tbody>
</table>

Tab. 1 shows the thiarubrine canal region identification accuracy expressed in the form of $F_1$-score and Jaccard indices. The reference segmentation results were obtained manually by experts for 4 slices randomly selected from the same image as the slice in Fig. 5. The process of manual segmentation was limited due to its time-consuming and required extensive work.

Tab. 2: The list of identification accuracy indices for the phantom ellipsoid region from Eqn. (7)

<table>
<thead>
<tr>
<th>case ID</th>
<th>$F_1$-score</th>
<th>Jaccard</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.981</td>
<td>0.962</td>
</tr>
<tr>
<td>2</td>
<td>0.980</td>
<td>0.961</td>
</tr>
<tr>
<td>3</td>
<td>0.986</td>
<td>0.972</td>
</tr>
<tr>
<td>4</td>
<td>0.987</td>
<td>0.974</td>
</tr>
<tr>
<td>5</td>
<td>0.981</td>
<td>0.962</td>
</tr>
<tr>
<td>6</td>
<td>0.982</td>
<td>0.963</td>
</tr>
<tr>
<td>7</td>
<td>0.979</td>
<td>0.959</td>
</tr>
<tr>
<td>8</td>
<td>0.986</td>
<td>0.973</td>
</tr>
<tr>
<td>9</td>
<td>0.982</td>
<td>0.965</td>
</tr>
<tr>
<td>10</td>
<td>0.985</td>
<td>0.973</td>
</tr>
</tbody>
</table>
The identification accuracy indices for ellipsoidal phantom objects were evaluated for 10 cases of different tissue background and region intensity patterns. The average values of $F_1$-score and Jaccard indices exceed 98% and 95.5% respectively. The automatic segmentation of real root region compared with experts segmentation gives accuracy listed in Tab. 1. In this case the average algorithm accuracies $F_1 \approx 92\%$ and $J \approx 90\%$ are lower than in the phantom case. This is mostly due to blurred or vanishing borders between the tested region and root tissue. The algorithm of proposed method has two important parameters adjusted experimentally:

1. the radius $R = 8 \text{ px}$ of the ball mask $M(p, R)$ applied in the region growing procedure,

2. the factor $k = 0.9$ in Eqn. (4) adjusting the upper intensity threshold $T$ of selected region.

The radius $R$ should be large enough so that the algorithm was not able to penetrate in the narrow passages between the canal and other image regions with similar grey-levels. when $R$ is too large the canal edges are not exactly mapped (over-smoothed).

The analysed hairy root image consists of 100 B-scans with a resolution of $600 \times 2048$ pixels, one illustrated in Fig. 5. The reduction of degraded line artefacts requires time $\approx 220s$, the time of region identification exceeds $\approx 75.5s$ and physical memory consumption is about 4.6 GB. 

![Fig. 5: Example OCT slices (a) original version, (b) with the identified root canal (bright)](image-url)
Fig. 6: Selected OCT phantom slices (a) ellipsoidal object with marked pixels, (b) with the result of segmentation

7 Conclusions

Due to their fast growth rates and biochemical stability, "hairy root" cultures remain unsurpassed as the choice for model root systems and have promise as a bioprocessing system. Using OCT, we are able to distinguish different layers and structures in the subsurface boundary regions of hairy plant roots. OCT scans seem to be more detailed and can be used for more adequate thiarubrine canals recognition, than traditional optical methods like microscope, which require sectioning of material and can destroy arrangement and composition of metabolites due to the long light exposure. The OCT method allows to preserve examined root for further cultivation and study. The proposed method for thiarubrine canal identification gave satisfactory results when tested for several B-scans verified by specialists. Thanks to the region growing approach the identification algorithm considers only canal pixels what accelerates its execution. Each of the tested OCT images is almost a billion pixels so their processing requires a lot of time and a large amount of operating memory. The authors managed to analyse only 100 B-scans; the analysis of whole images required more than 32GB RAM. In the future, to accelerate the method artefact reduction will be developed in C++ and the canal identification should be parallelized using GPU cores.

References


Coherence Tomography: Technology and Applications, 919–959


