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Determination of collagen content within picrosirius red stained paraffin-embedded tissue sections using fluorescence microscopy

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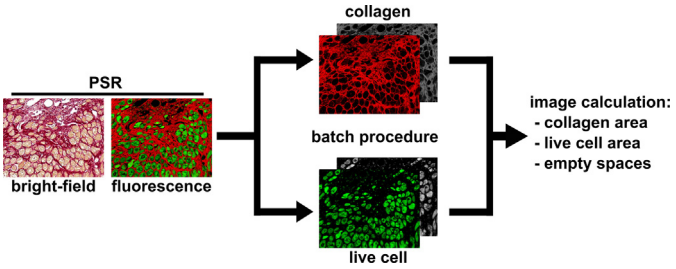
GRAPHICAL ABSTRACT



ABSTRACT

Picrosirius red (PSR) staining is a commonly used histological technique to visualize collagen in paraffin-embedded tissue sections. PSR stained collagen appears red in light microscopy. However it is largely unknown that PSR stained collagen also shows a red fluorescence, whereas live cells have a distinct green autofluorescence. Both emission patterns can be detected using standard filter sets as found in conventional fluorescence microscopes. Here we used digital image addition and subtraction to determine the relative area of the pure collagen and live cell content in heart tissue in a semi-automated process using standard software. This procedure, which considers empty spaces (holes) within the section, can be easily adapted to quantify the collagen and live cell areas in healthy or fibrotic tissues as aorta, lung, kidney or liver by semi-automated planimetry exemplified herein for infarcted heart tissue obtained from the mouse myocardial infarction model.

- Use of conventional PSR stained paraffin-embedded tissue sections for fluorescence analysis.

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- PSR and autofluorescence images are used to calculate area of collagen and area of live cells in the tissue; empty spaces (holes) in tissue are considered.
- High throughput analysis of collagen and live cell content in tissue for statistical purposes.

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Method details

Picrosirius-red (PSR) is a well-known method to stain collagen in histology. However it is rather unknown that PSR stained tissue sections can also be analyzed brilliantly by fluorescence. Here we provide an easy and fast procedure how to determine collagen and live cell content for descriptive or statistical purposes from PSR stained tissue sections using fluorescence microscopy, semi-automated image processing and standard software.

Required equipment and software

- PSR stained paraffin-embedded tissue section (here: heart) [5].
- Epifluorescence microscope (here: Zeiss Z1m Imager) with standard filter sets capable to detect FITC and Rhodamine (here: "FITC": Ex: 450–490 nm, Em: 500–550 nm, "Rhodamine": Ex: 538–562 nm, Em: 570–640 nm).
- Fluorescence camera with appropriate acquisition software (here: Axiocam MRm with Axiovison 4.8) capable of producing 8-bit images [jpg or a proprietary (batch) convertible file format]. A confocal microscope can be used as well as described [1], but is not necessary.
- Zeiss ZEN lite 2011 SP1 1.0.1.0 (freeware, Zeiss, Oberkochen, for batch extraction of *.jpg channels from *.zvi files).
- Adobe Photoshop 7.0 or newer (for batch image downscaling from color to 8-bit grayscale images, image calculation and processing).
- DirPrintOK (freeware, for file name inclusion to MS Excel, www.softwareok.de).
- MS Excel 2010 or similar program (for data acquisition and calculation).

The method here is described for the use with a Zeiss Z1m Imager epifluorescence microscope, which results in the acquisition of a proprietary *.zvi file which contains the actual fluorescence images channels and metadata. However any other fluorescence microscope can be used if images can be acquired or finally converted to individual 8-bit jpg channel files. It is noteworthy that freeware software as ImageJ or CellProfiler instead of Abode Photoshop might also be capable of performing the procedures presented here.

Image acquisition

1. Carefully choose the field of view according to your experimental design. Avoid field of views with artefacts due to tissue cutting or incorrect attachment of the section to the slide. Always use the same magnification for all image acquisitions (recommended: 20× objective).
2. Set up exposure times for autofluorescence (green) and collagen (red) channels. Do not acquire other channels than the red and green fluorescence channel since this will increase data amount and delay the following processes. Use a range indicator in your acquisition software. Do not overexpose green and red fluorescence channels. For correct determination of collagen content make sure the image is focused to the (red) collagen channel, especially tissue sections >7 μm. Focusing to the (green) autofluorescence channel is not recommended. The green channel seems to be out of focus from time to time compared to the collagen scaffold probably due to the fixation, embedding and cutting procedure.