A novel image analysis algorithm for scratch area determination in a scratch assay.

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Introduction

- Scratch area determination is crucial for data obtention after a scratch assay
- Manually it is time-consuming and challenging
- Automatization tools are available but
  - Expensive
  - Limited in their performances
  - Or not up to date
- We developed **SAMScratch** (Systematic Area Measurement of Scratch)
  - Available as a MATLAB™ standalone application
  - Detects the scratch and computes its area (Fig. 1)
  - Allows fine tuning of the process by user
  - Computes the migration speed if the images belong to a time series (Fig. 1)

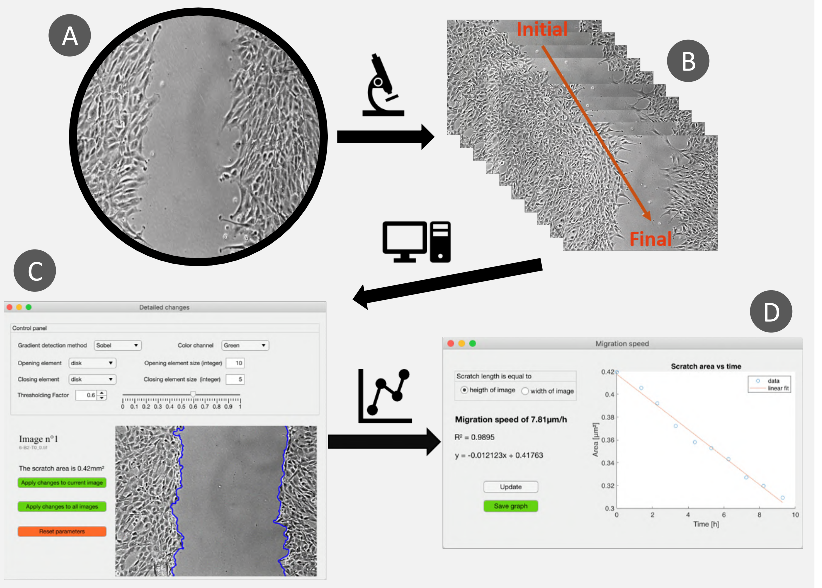

Fig. 1. Use of SAMScratch for migration speed computation after a scratch essay. A) Scratches are photographed with bright field microscopy. B) One photo is taken every hour to monitor cell migration. C) Images are processed by SAMScratch to measure the area of the scratch. D) SAMScratch computes the migration rate.

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App overview

1. **Browse…** button allows to select a directory.
2. **Go** button launches an analysis.
3. Arrows allows you to move through the analyzed images.
4. a and b. Slider and box allow to easily change the result segmentation of the scratch by modifying the thresholding factor (TF). c. thresholding factor can be changed for all images together through **Apply to all images** button.
5. More parameters of the algorithm can be changed through **details** button.
6. Changes can be cancelled through the **reset analysis** button.
7. Results can be saved in an excel or text file through **save results** button.
8. Segmented images can be saved through **save images** button.
9. When an analysis is performed, the next one can be started through the **new analysis** button.

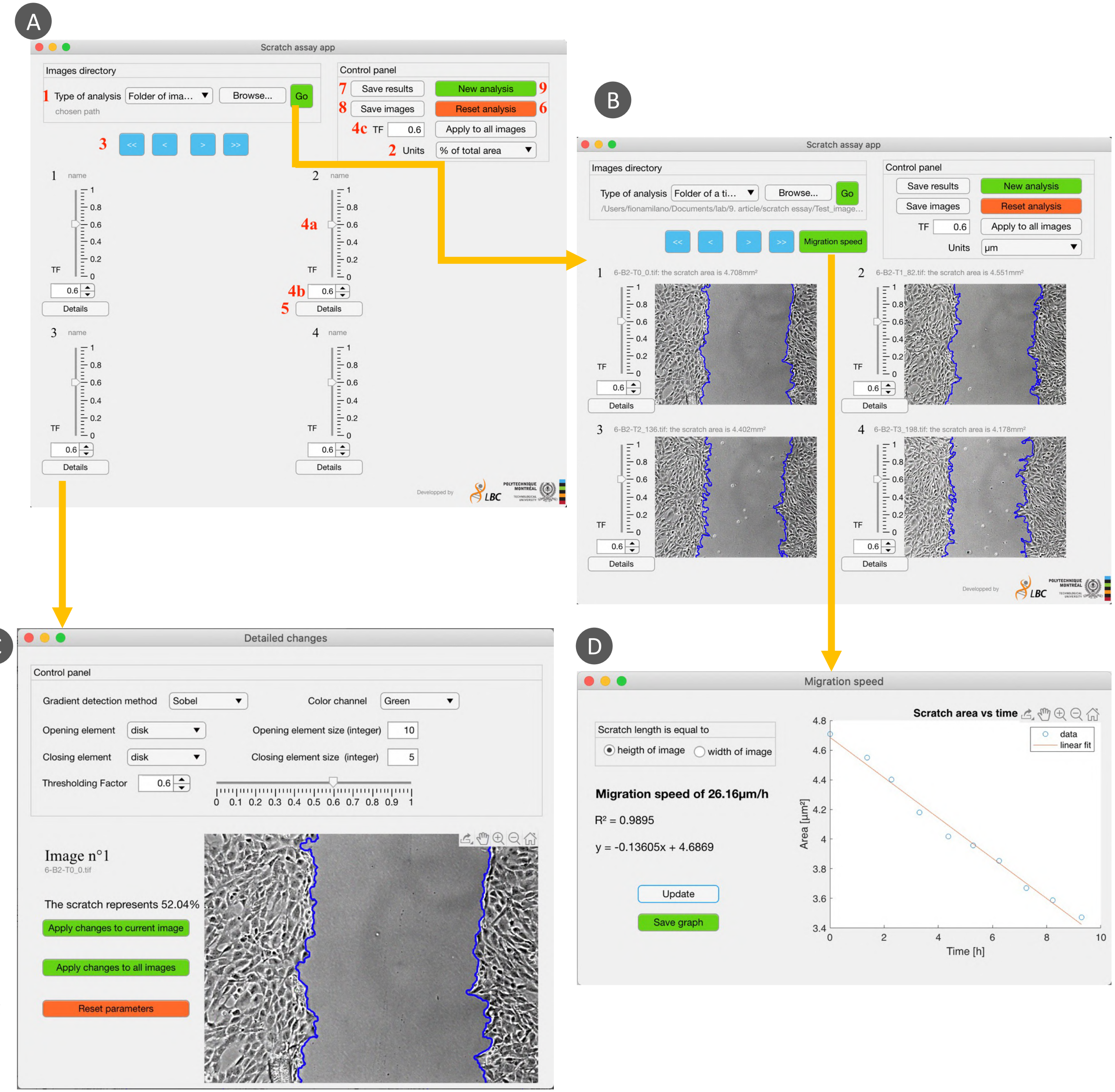

Fig.2. A) Main app window. B) Main window after launching the analysis. C) Detailed change panel allows to fine tune the scratch detection if needed. D) In case of time series, SAMScratch computes the migration speed.

Materials and methods

- Fig. 3 presents a flowchart of the algorithm
- SAMScratch's reliability is quantified using:
  - The relative error $\mathrm{RE} = \left|\frac{A_{GT}-A_{SA}}{A_{GT}}\right| = \left|\frac{FN-FP}{N_{GT}}\right|$
  - The misclassification rate $\mathrm{MR} = \frac{FP+FN}{T}$

where
- A_{GT} = ground truth = area derived from a manual segmentation
- A_{SA} = area computed by the studied algorithm
- FN = number of false negative pixels
- FP = number of false positive pixels
- N_{GT} = number of pixels belonging to the scratch according to ground truth
- T = total number of pixels in the image

- Reliability computed for 35 variate images
- Based on those images SAMScratch is compared to three other algorithms :
  - Tscratch, a free MATLAB application
  - A method proposed by Jonkman *et al.* implemented as an ImageJ macro
  - Wimscratch, a commercial software

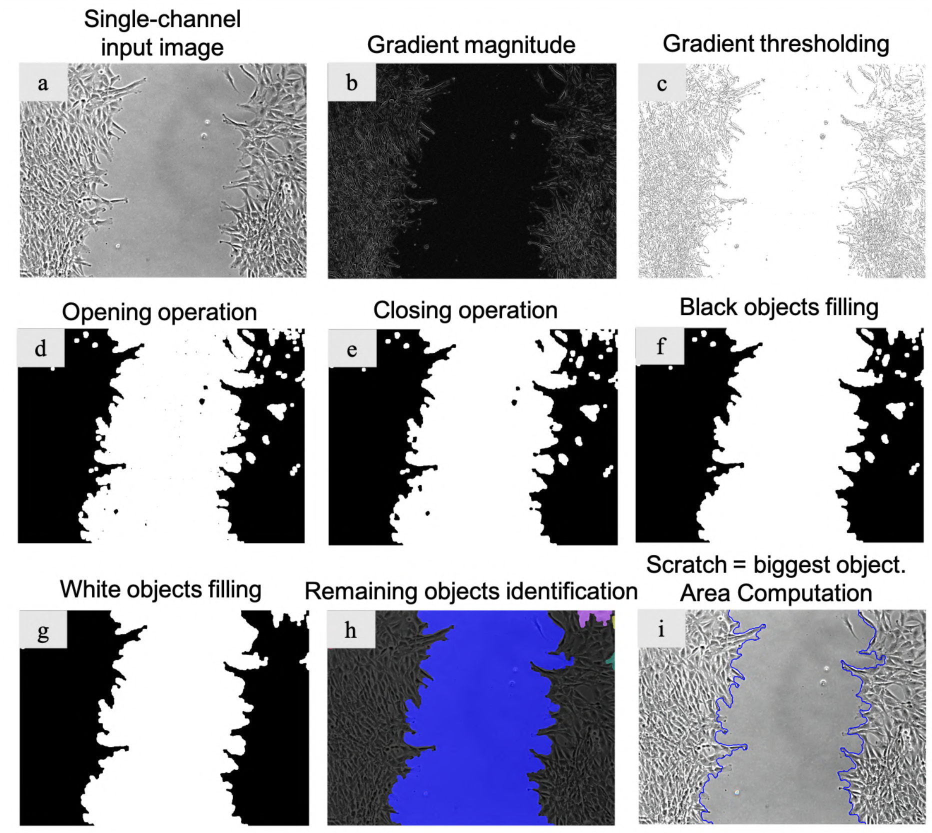

Fig. 3. Flowchart of the SAMScratch algorithm

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Results

- Table 1. presents mean and median RE and MR for the 4 compared algorithms
  - SAMScratch presents the smallest average and median RE and MR
- Fig. 4 shows the scratch segmentation result obtained by the 4 studied algorithms on 6 images

Method	Relative Error		Misclassification Rate	
	Average	Median	Average	Median
SAMScratch	3.9%	2.3%	3.7%	3.8%
TScratch	8.1%	4.0%	6.8%	6.3%
Image J macro	74.5%	20.0%	/	/
WimScratch	11.3%	8.6%	4.9%	4.4%

Table 1. Average and median RE and MR for the four studied algorithms for scratch identification. Image J macro shows big relative errors. The misclassification rate is therefore not computed for the Image J macro as it would obviously lead to a high value too.

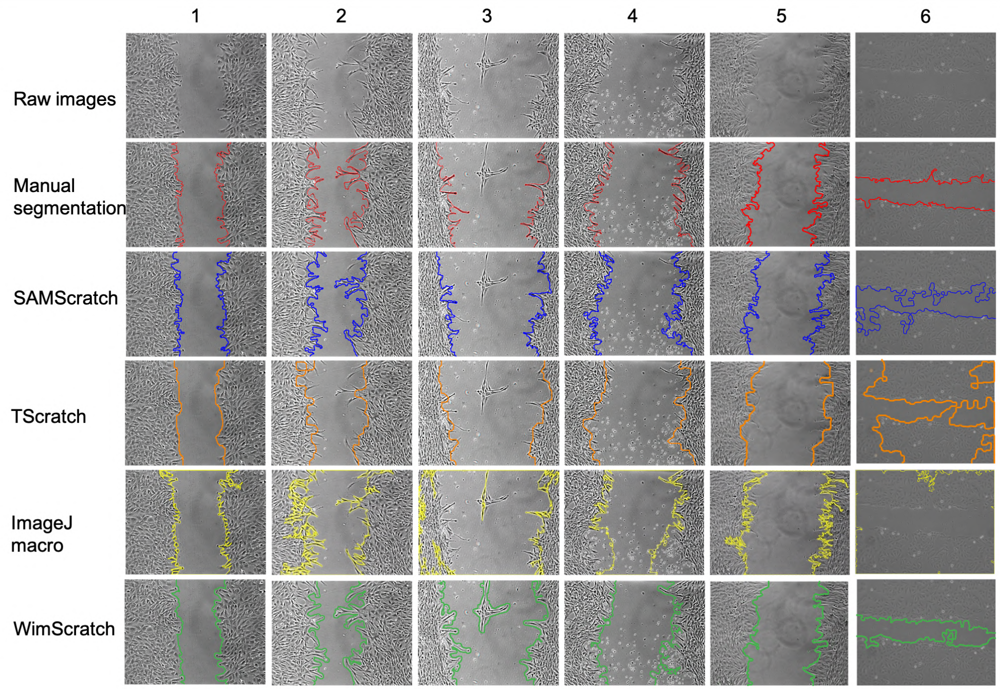

Fig. 4. Comparative segmentation results for 5 methods on 6 representative images: (1) Typical image obtained during a scratch assay with moderate migration; (2) Typical image obtained at the end of a scratch assay with high migration rate and individual cells extended; (3) Undesired cluster of cells in the middle of scratch; (4) Undesired dead cells and debris in the scratch; (5) Typical image obtained during a scratch assay with low contrast; (6) Typical image obtained during a scratch assay with extremely low contrast.

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Discussion and conclusions

- RE and MR show that SAMScratch is the most precise of the four algorithms (for studied images)
- Compared to other algorithms, SAMScratch performs well at:
    - Segmenting long cell extensions
    - Segmenting low contrast images
    - Rejecting undesirable clusters of cells in the scratch + rejecting dead cells and small debris
- Main limitations:
    - Does not discriminate entirely successfully the cell sheet from adjacent dead cells or debris (e.g. Figure 4)
    - Fails to precisely handle extremely low contrast images
    - Depends on MATLAB™

The software is freely available as well as further instructions of use (https://github.com/Biomaterials-and-Cartilage-Laboratory/SAM-Scratch).

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