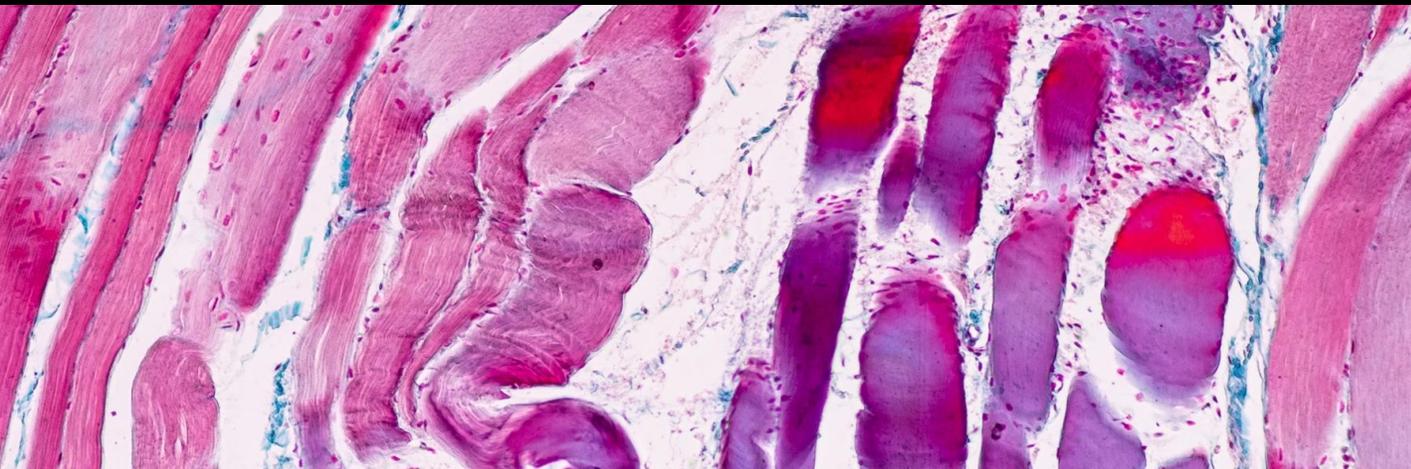


# The Science of Tissue Processing Tidal vs Rotational Processing

Andrew R. Lisowski, MS, HTL (ASCP)  
*Sr Technical Content Marketing Manager*



# WHAT IS TISSUE PROCESSING?

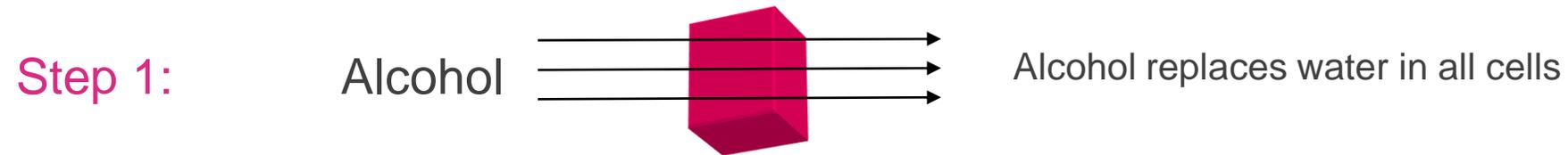
- Tissue processing is a procedure of removing water from cells and replacing it with a medium which solidifies allowing thin sections to be cut on a microtome.
- Once tissue is properly fixed it goes through a process which involves the following steps:
  - **Dehydration**
  - **Clearing**
  - **Infiltration**
- Tissue processing is routinely done on an instrument called a Tissue Processor.



# TISSUE PROCESSING: OVERVIEW

“Tissue processing” describes the steps required to take animal or human tissue from fixation to the state of complete infiltration with a histological paraffin.

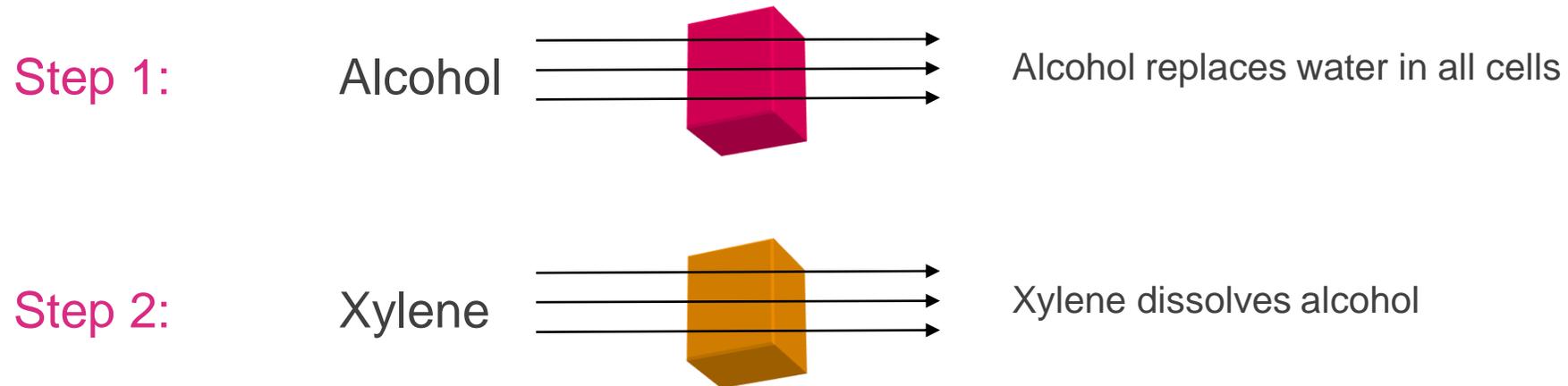
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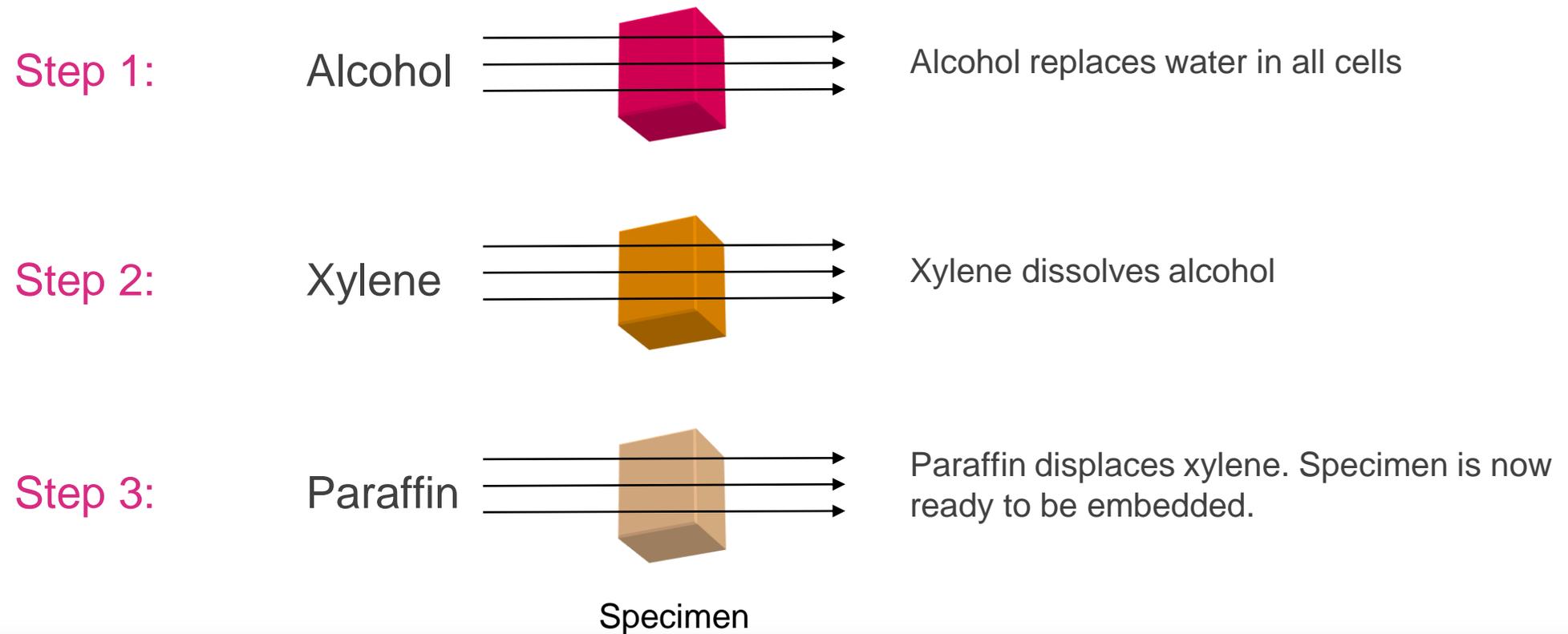
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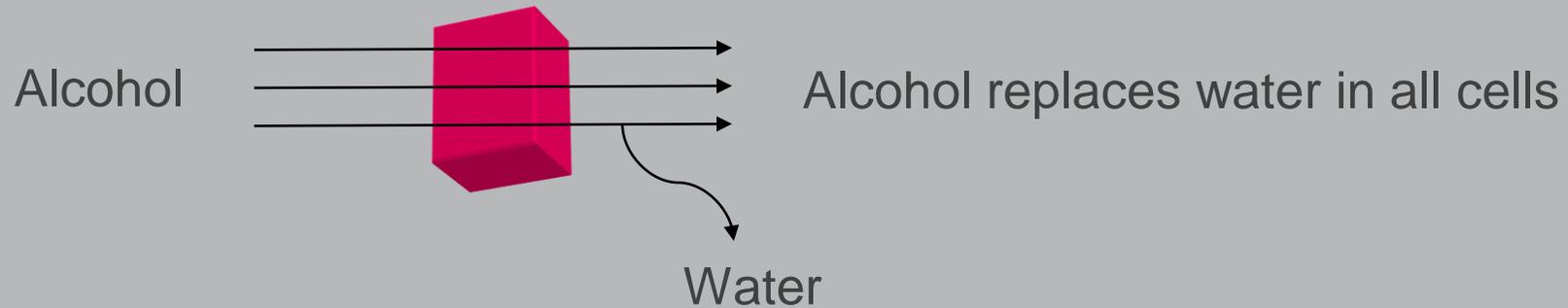
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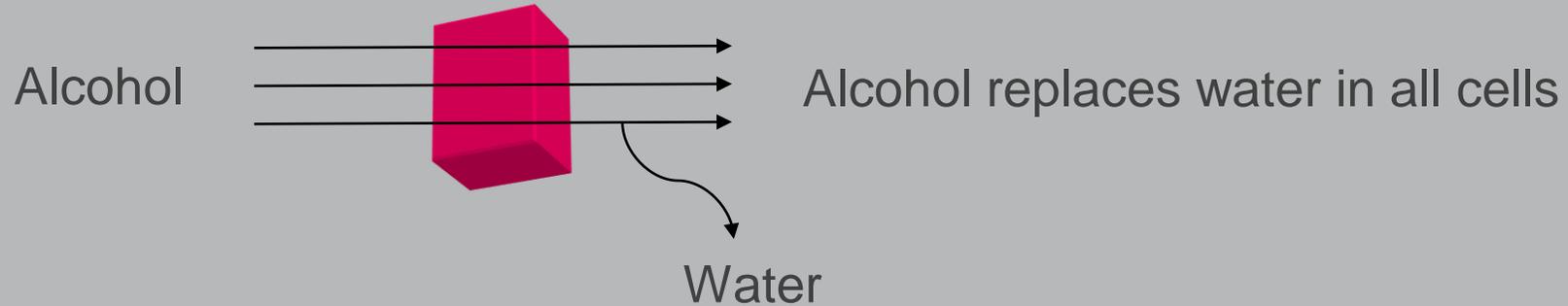


# STEP 1: DEHYDRATION



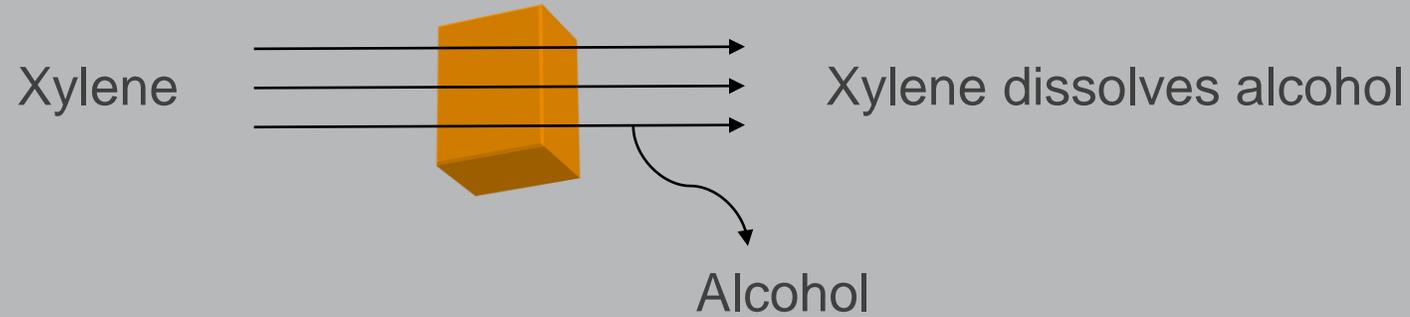
- Since paraffin is hydrophobic (immiscible i.e., not mixable with water), water inside a specimen must be removed before it can be infiltrated with paraffin. This process is carried out by immersing specimens in a series of alcohol.

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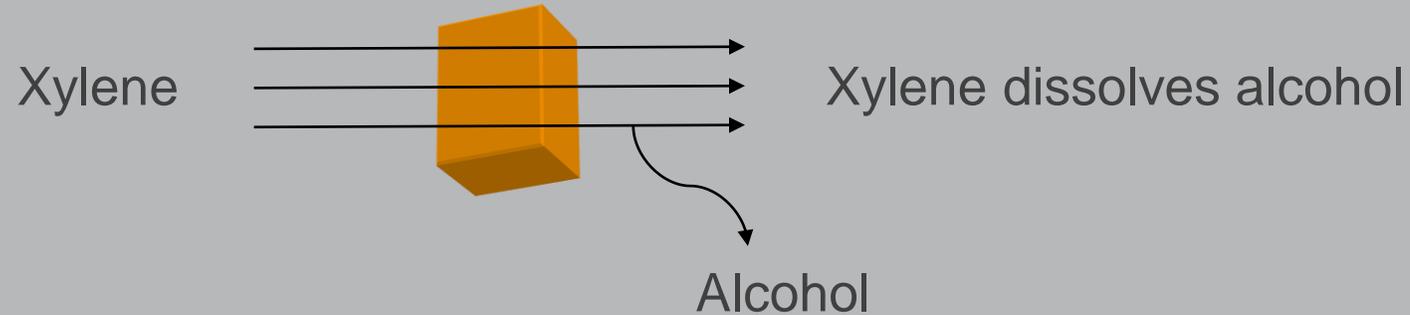
- Since paraffin is hydrophobic (immiscible i.e., not mixable with water), water inside a specimen must be removed before it can be infiltrated with paraffin. This process is carried out by immersing specimens in a series of alcohol.
- Alcohol **progressively replaces** water in all the cells of the specimen.
- A series of increasing (typically from 70% to 100%) alcohol concentrations are used to avoid excessive distortion of the tissue.

## STEP 2: CLEARING



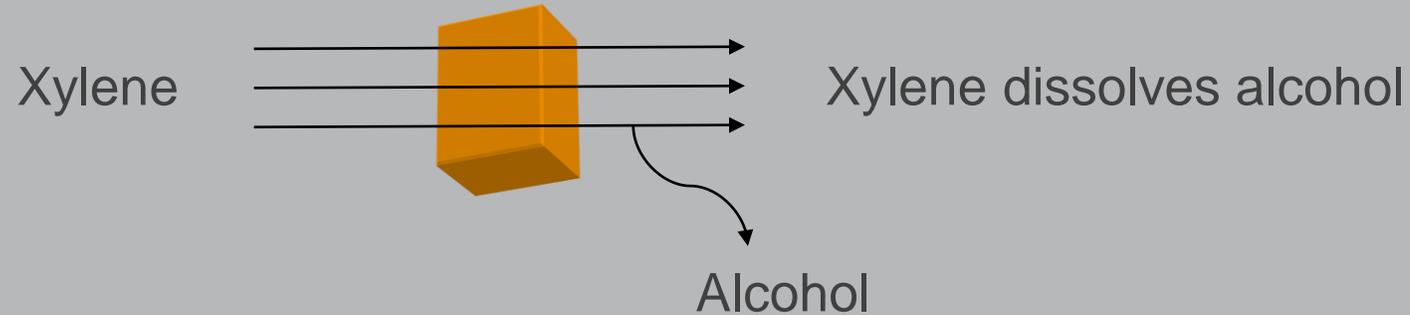
- Since alcohols and paraffins are not miscible, an intermediate solvent that is fully miscible with both (such as xylene), must be used.
- This solvent displaces the alcohol in the tissue through the process called “clearing”.

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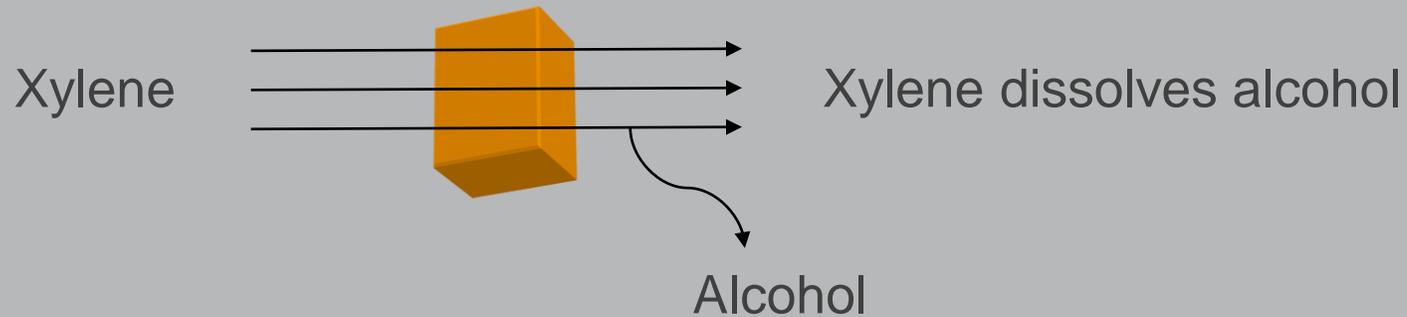
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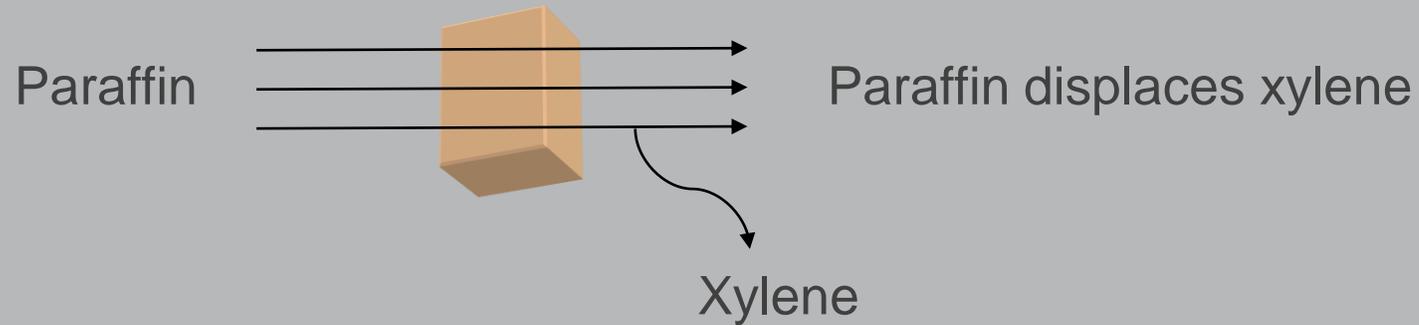
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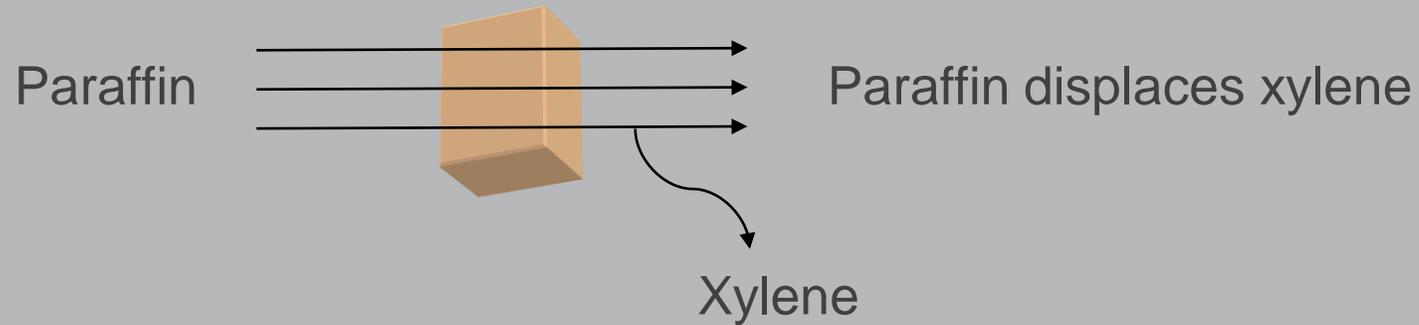
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- Another important role of the clearing agent is to remove a substantial amount of fat from the tissue which otherwise presents a barrier to paraffin infiltration.
- To make sure that all traces of alcohols are removed from tissues being processed, **multiple** changes of fresh, clear of carried-over alcohol, are required.

# STEP 3: INFILTRATION



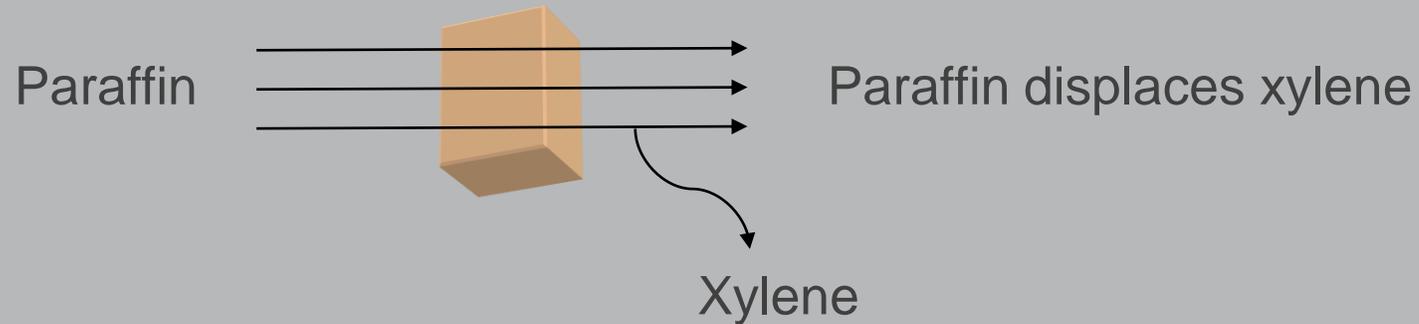
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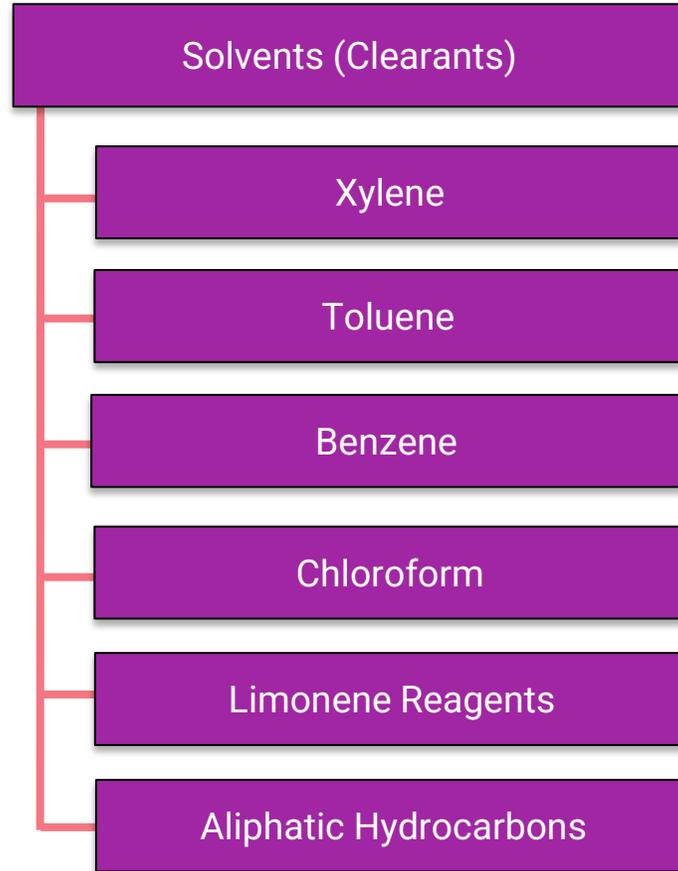


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- The amount of structural support given by solidified paraffin can be regulated by choosing different paraffin formulations
- **Multiple** changes of histological paraffin are required to completely displace the clearing agent.
- Paraffin infiltration is greatly enhanced by vacuum.

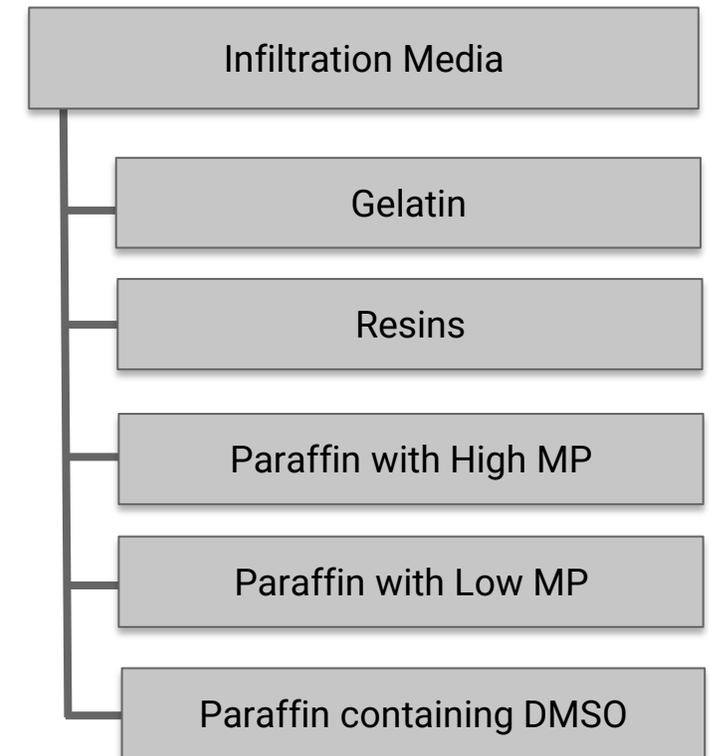
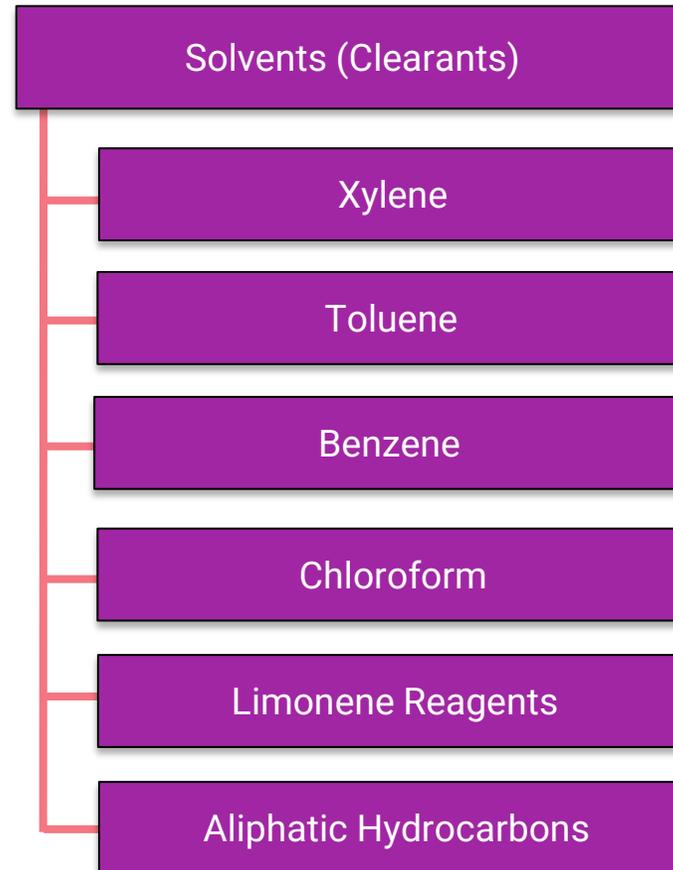
# COMMON PROCESSING REAGENTS



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# PROCESSING METHODS

Automated

Manual

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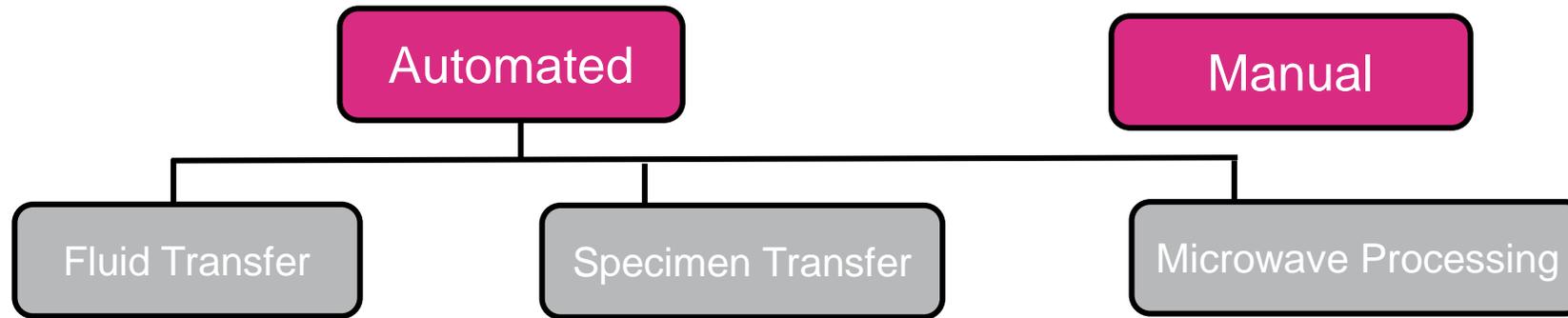
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## Manual Processing

- Slow, most labor-intensive method since transfer of specimens or changing reagents is done by hand. With an advance of automation this method is almost obsolete.

# PROCESSING METHODS

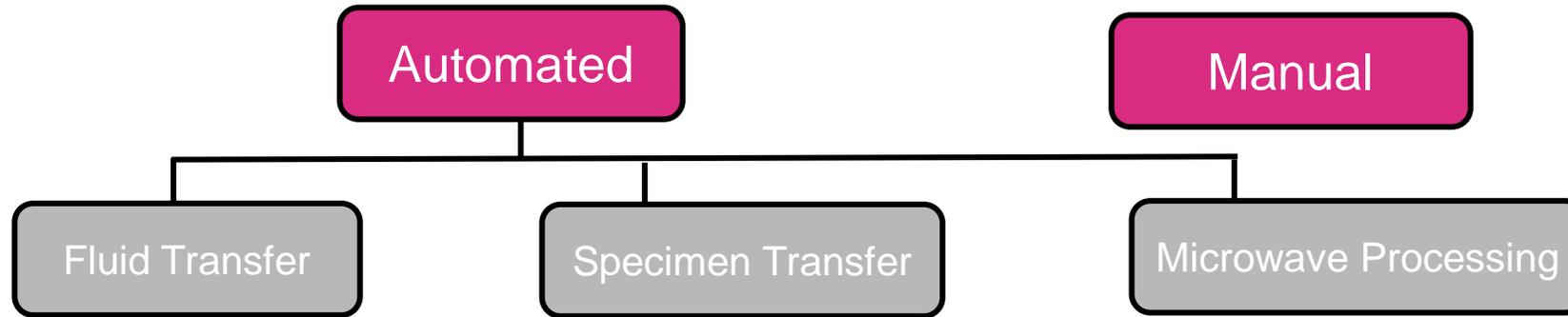


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# PROCESSING METHODS



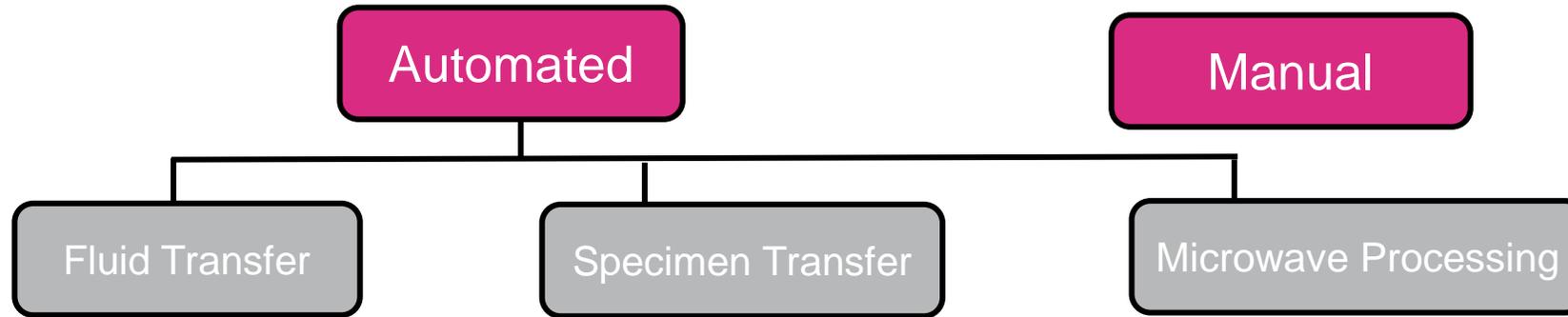
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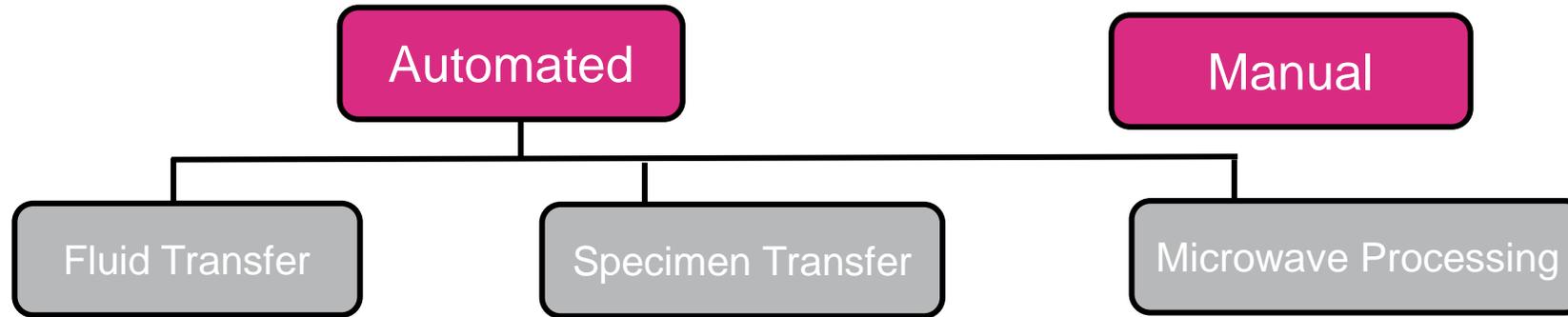
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# PROCESSING METHODS



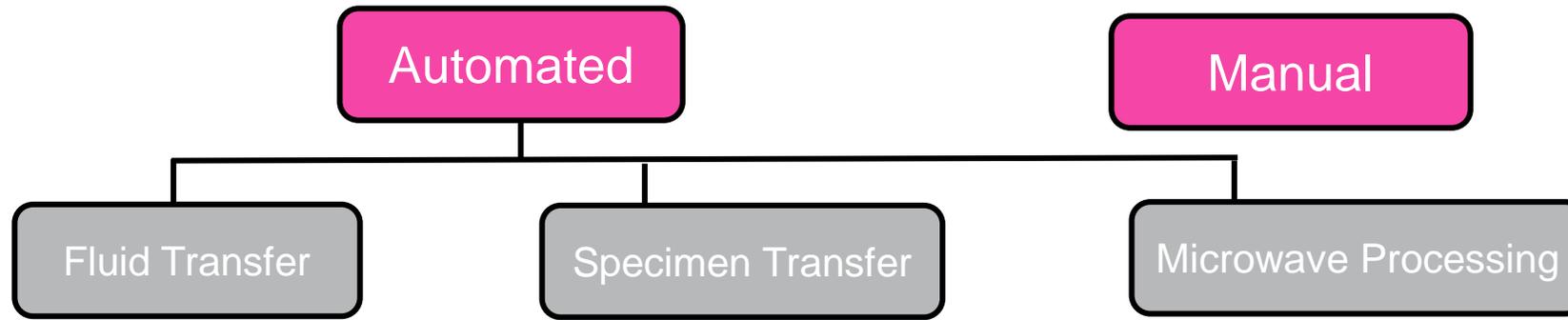
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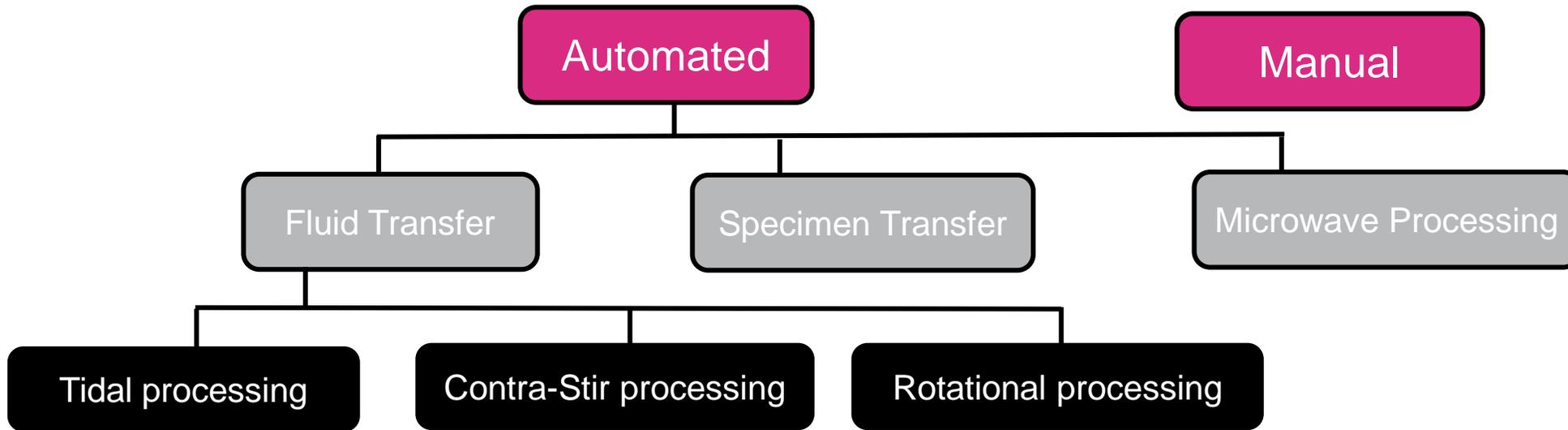
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- **Microwave** assisted processing accelerates processing by heating reagents.

# PROCESSING METHODS



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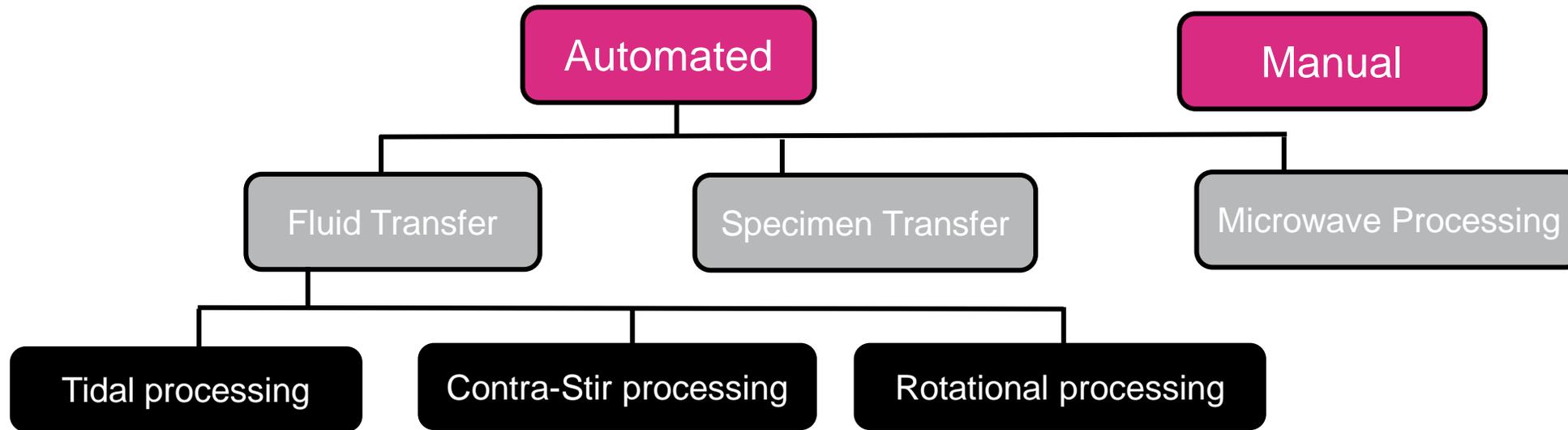


# POLL QUESTION #1

## **How familiar are you with Rotational Processing?**

- A. I've never heard of rotational processing.
- B. I'm somewhat familiar/I've heard of rotational processing before.
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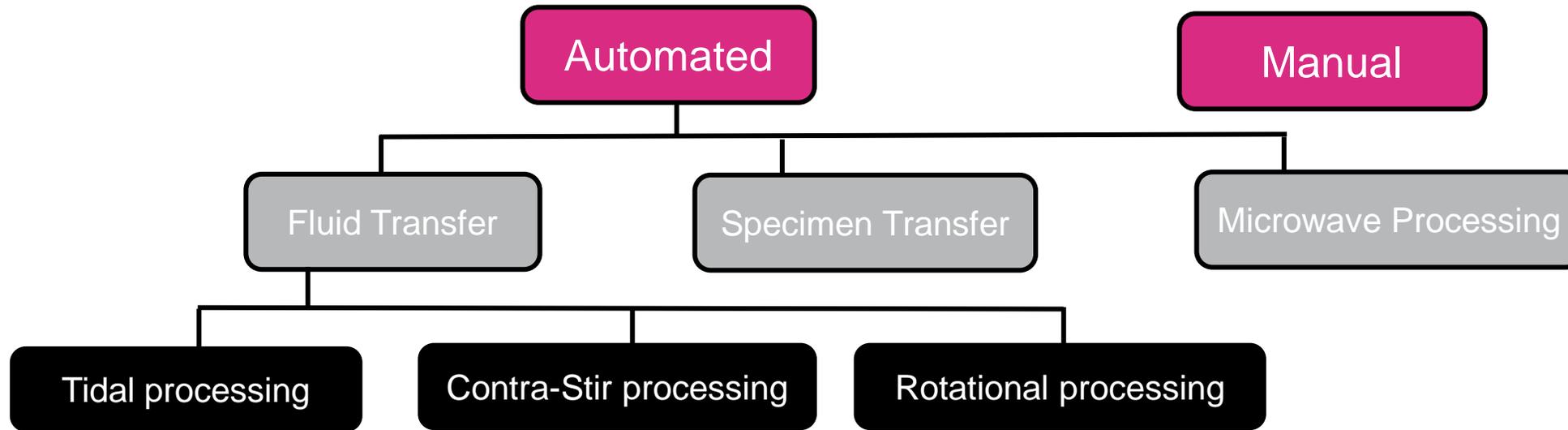
# PROCESSING METHODS



## Tidal Processing

- Tissue processing utilizing simple immersion and agitation techniques.

# PROCESSING METHODS



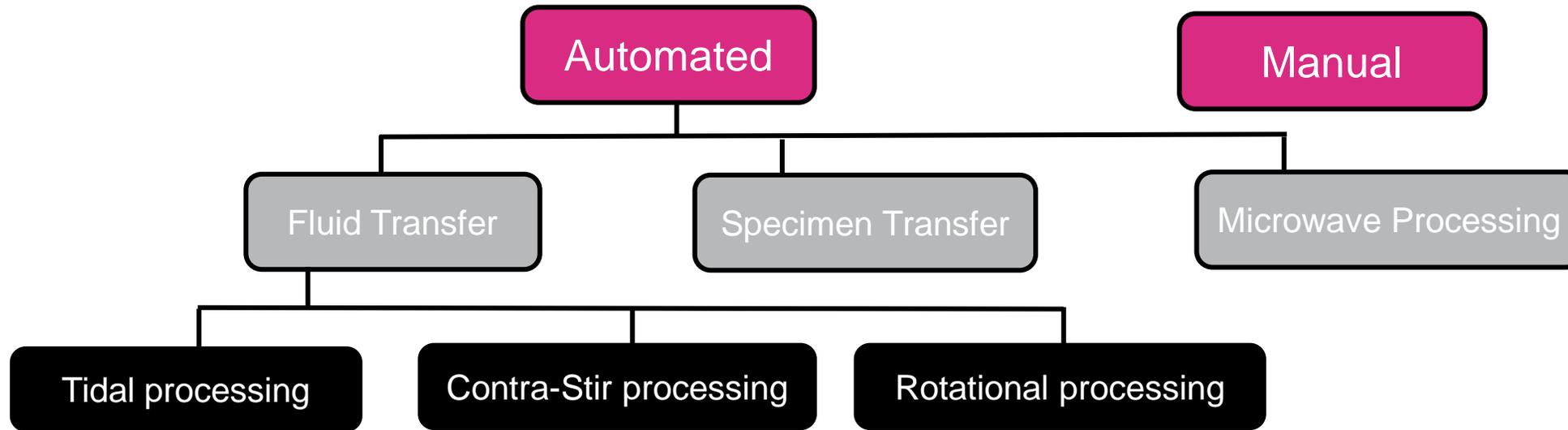
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- Cassette baskets are gently rotated inside the circular chamber, providing more effective agitation than an impeller.

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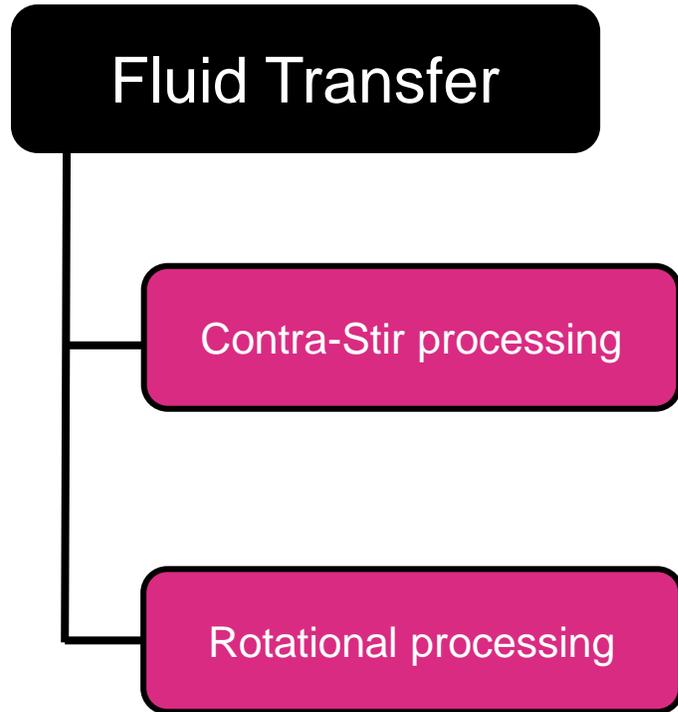
## Contra-Stir Processing

- Cassette baskets are gently rotated inside the circular chamber, providing more effective agitation than an impeller.

## Rotational Processing

- Rotational tissue processor utilizes a rotating rack to induce interaction between the fixative fluids and the tissue samples.

# TISSUE PROCESSOR – AGITATION MODES



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## Fluid Transfer

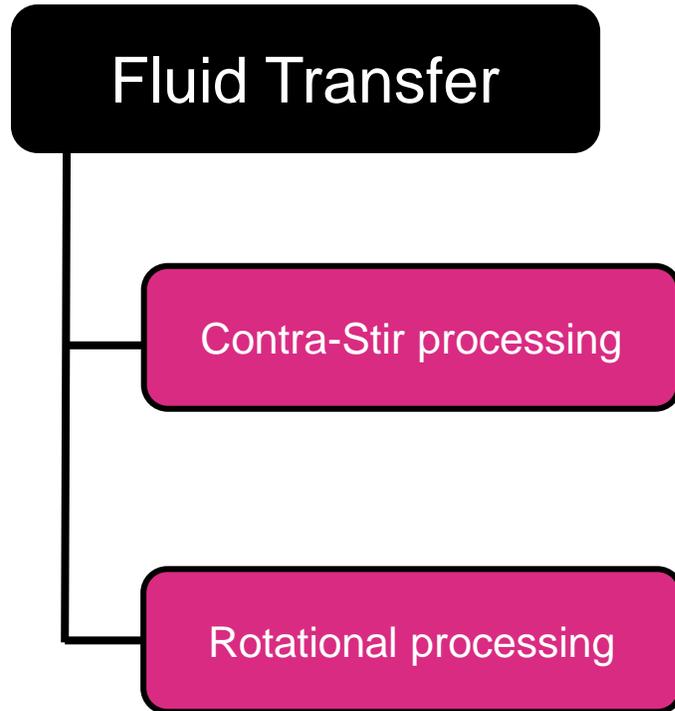
```
graph TD; A[Fluid Transfer] --- B[Contra-Stir processing]; A --- C[Rotational processing];
```

Contra-Stir processing

Uses **contra-stir agitation** which moves the baskets through the fluid on a central pivot rather than using an impeller to circulate the fluid. It moves the fluids  $1\frac{1}{4}$  turns one way and then reverses the direction.

Rotational processing

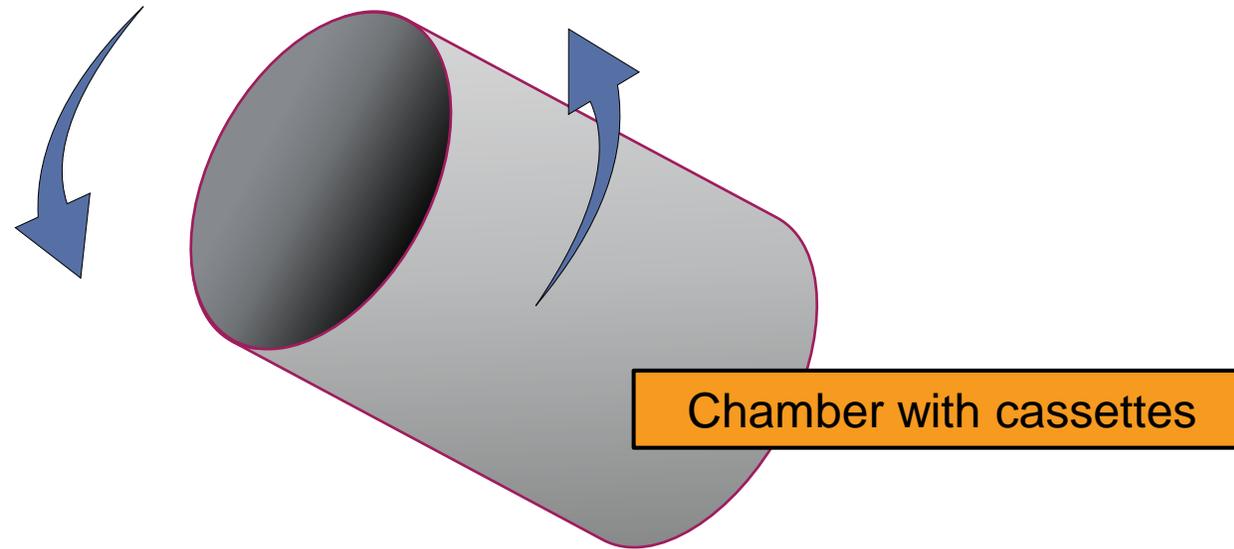
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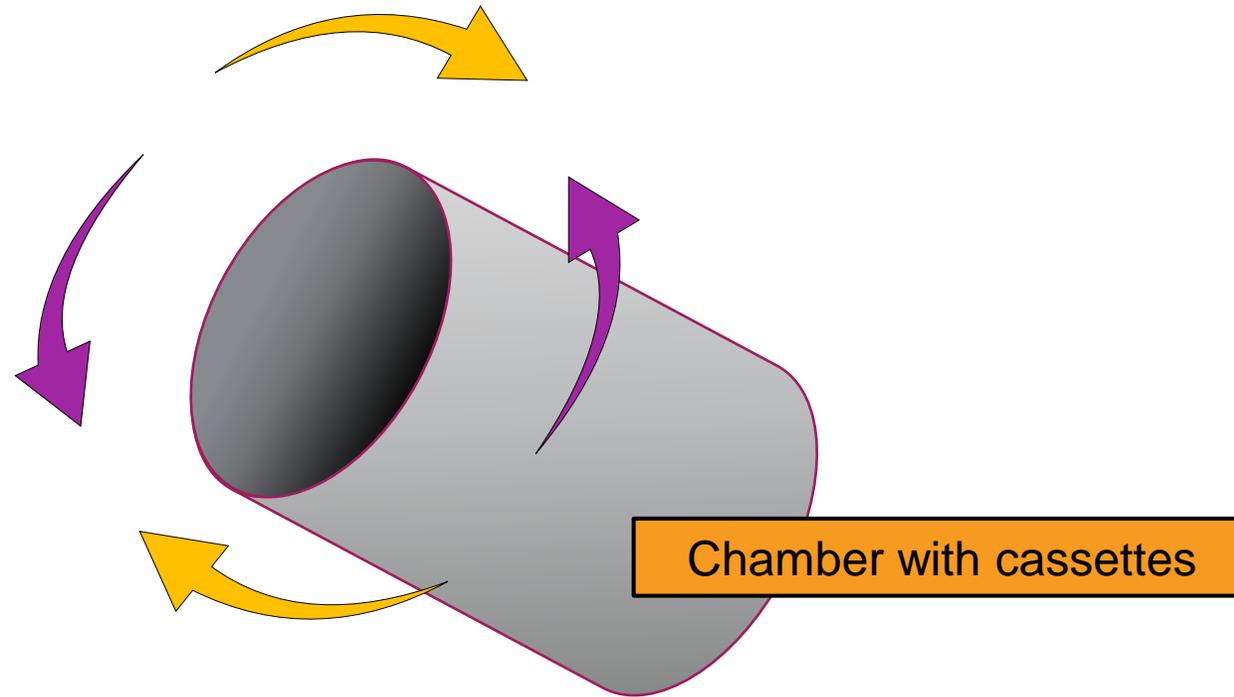
**Uses rotational chamber agitations**  
Rotational Tissue Processor (RTP) moves the baskets in a circular motion around a central cone and instead of immersed all the time the baskets move through the fluid. Tilted chamber configuration allows fluid movement in and out of the cassettes.

# ROTATIONAL ACTION



1. Instrument revolves the chamber in one direction

# ROTATIONAL ACTION



1. Instrument revolves the chamber in one direction
2. Next, it changes the revolutions into the opposite direction

## **How familiar are you with Rotational Processing?**

- A. I've never heard of rotational processing.
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## POLL QUESTION #2

**Which tissue type(s) gives you the most problems when processing (choose all that apply)?**

- A. Uterus
- B. Breast biopsy
- C. Decalcified ossified tissue
- D. Skin

# BASKET DESIGN ALSO AFFECTS TISSUE PROCESSING

Baskets with spaces in between individual cassettes provide better tissue accessibility to all processing reagents.

This translates into an increased flow volume and speed, better drainage rate and or an overall better improved fluid exchange.



Rectangular basket for tidal processor



Basket designed for a contra-stir processor



Basket designed for a rotational processor

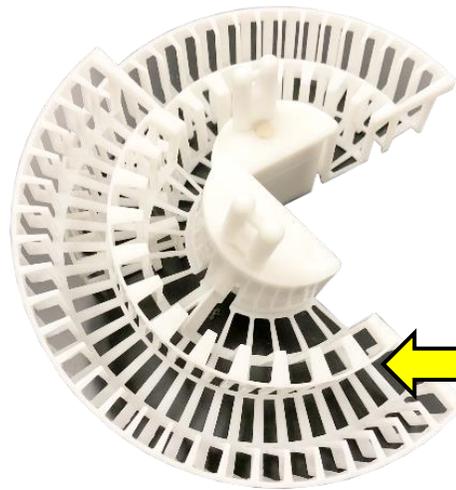
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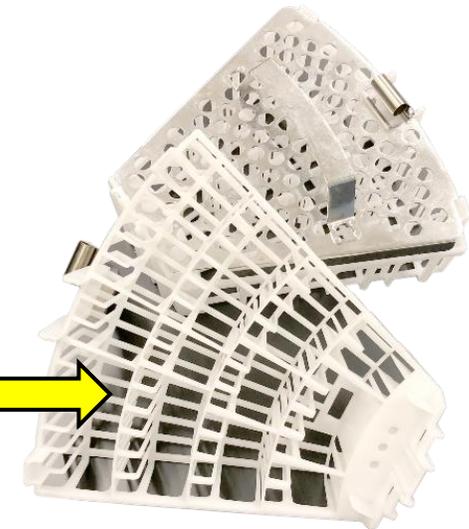


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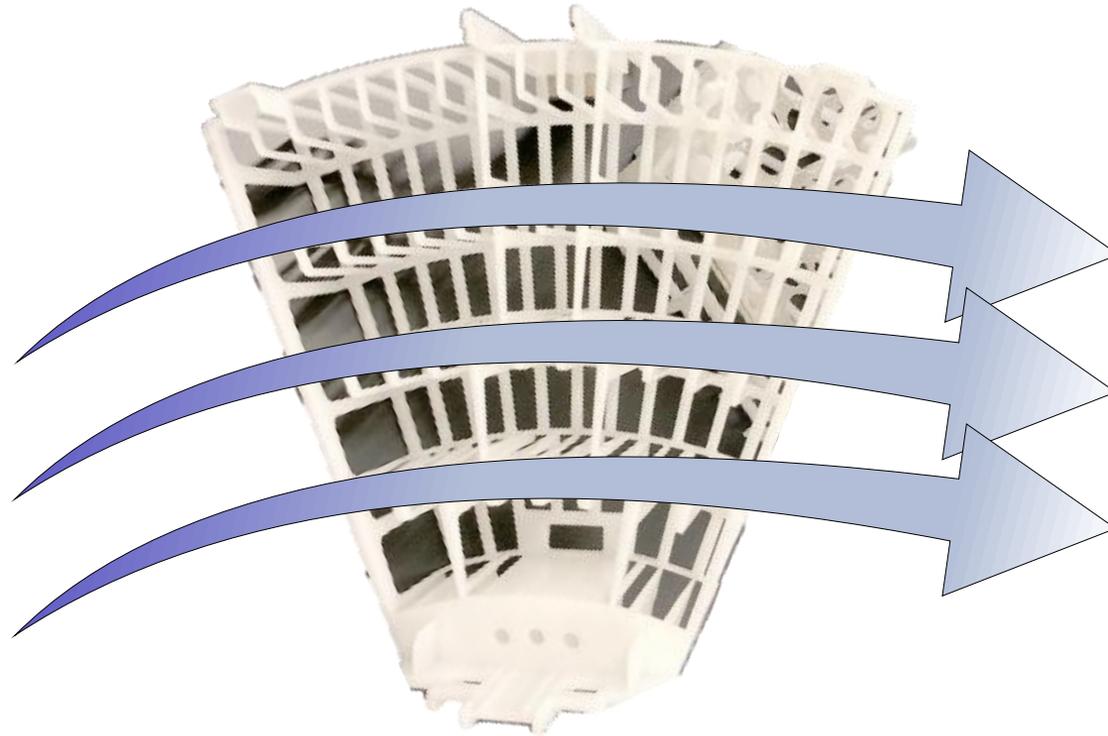
Spaces between cassettes



Basket designed for a rotational processor

# ENHANCED REAGENT FLOW

Thanks to a unique basket design (spaces between cassettes) and increased reagent movement (rotational processing), tissue samples are processed in less time. Additionally, thanks to a faster drainage, reagents carry-over is reduced.



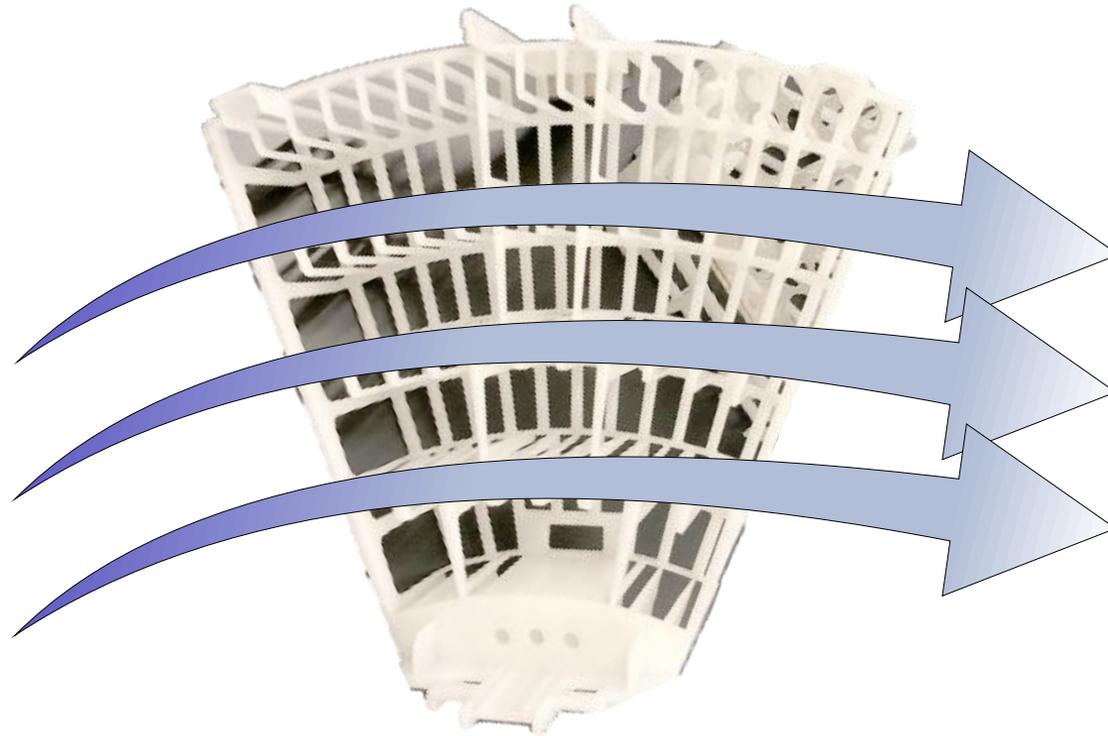
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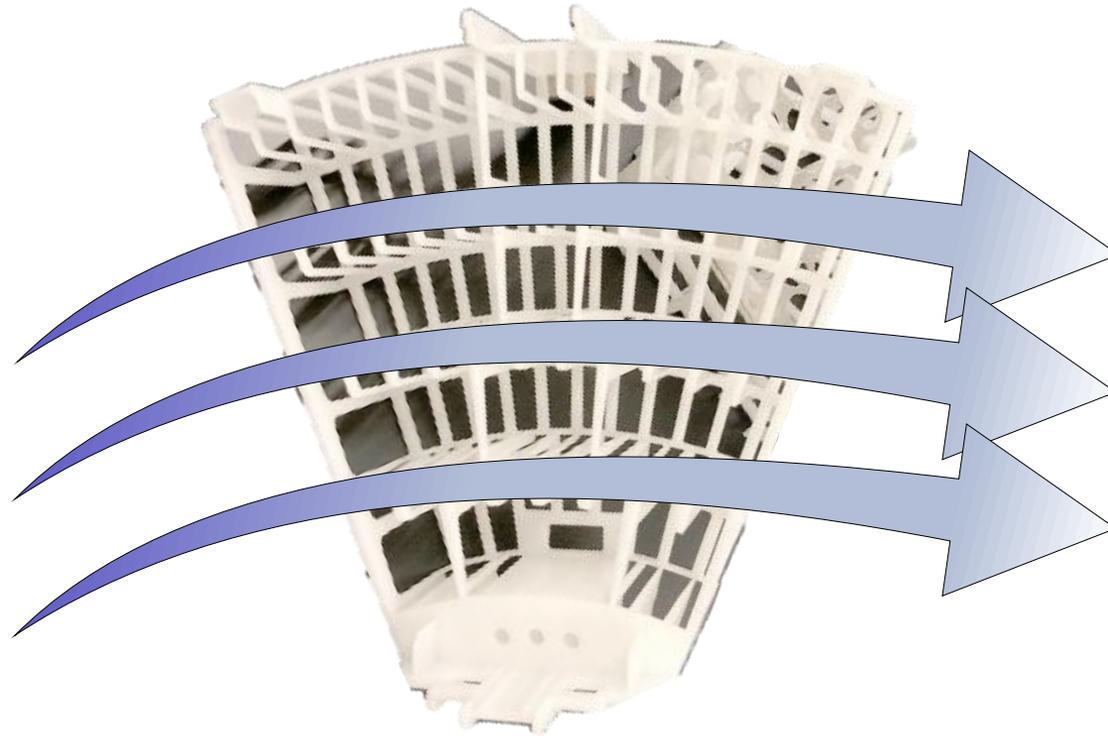
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## POLL QUESTION #2 - REVIEW

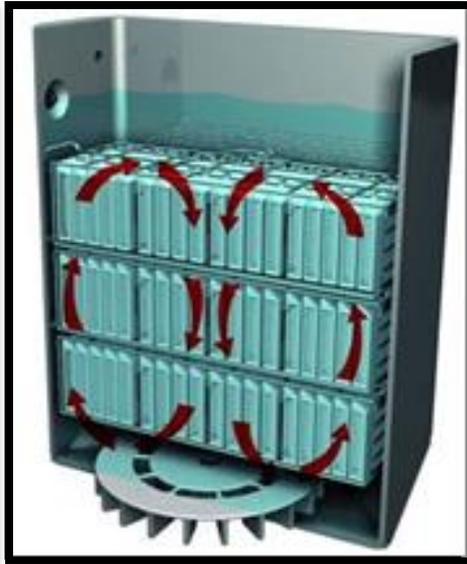
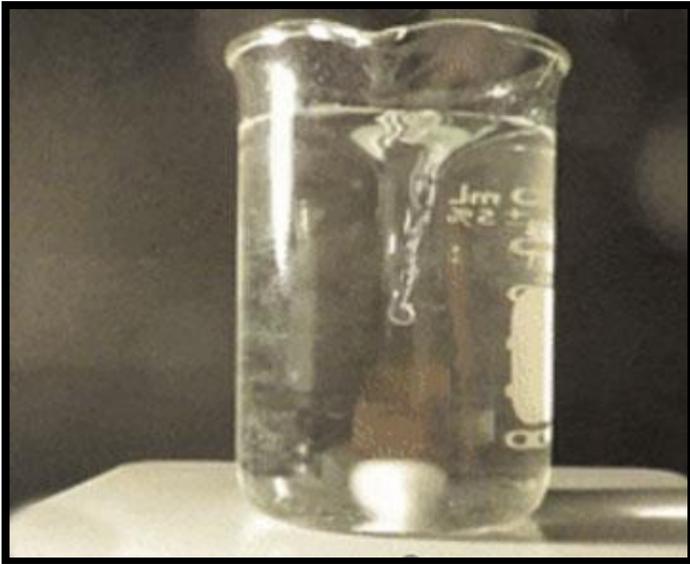
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# WHAT IS TIDAL AGITATION PROCESSING

Traditional processing utilizes simple tissue immersion combined with gentle agitation. It is frequently termed Tidal Agitation Processing.

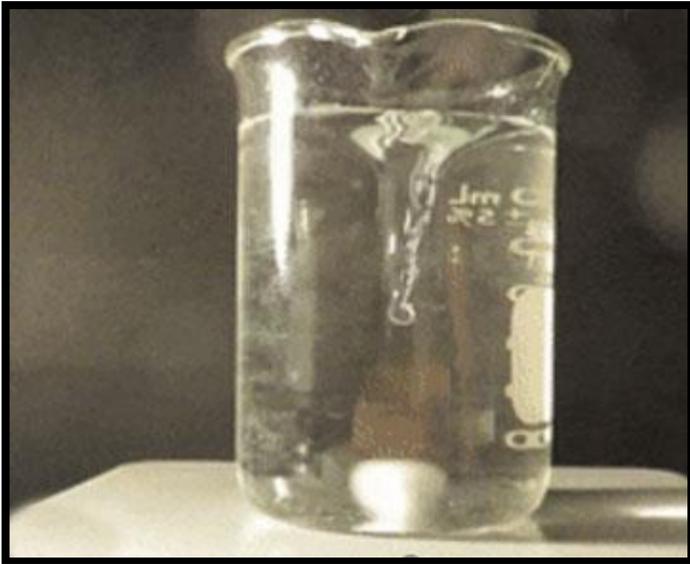
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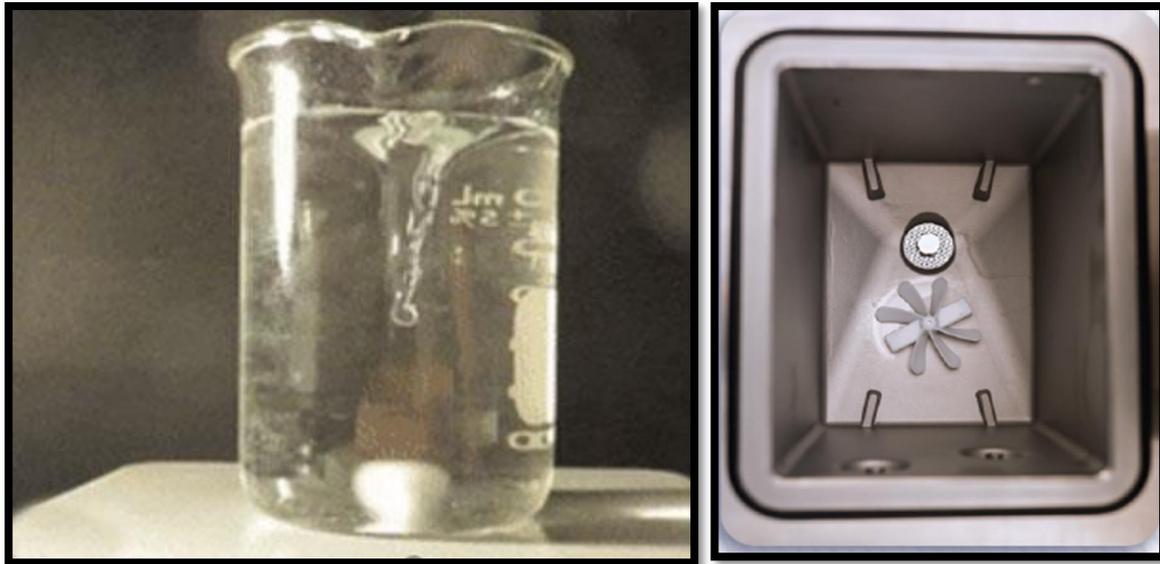
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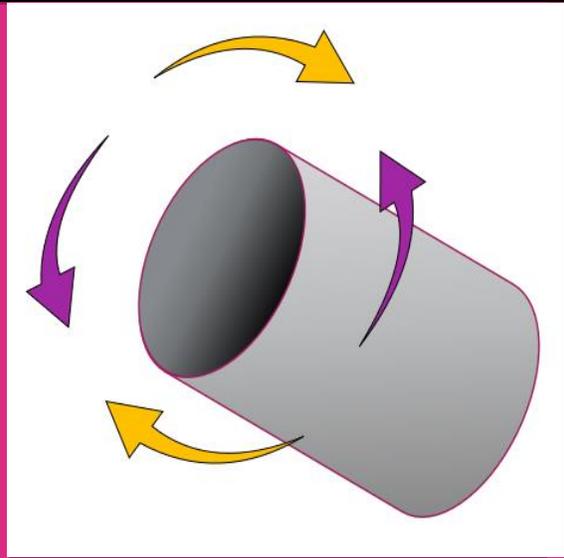
Vacuum-pressure cycles coupled with heated reagents might allow reduced processing times and improved infiltration of dense tissues.

However, it must be used cautionary as **higher temperatures can adversely affect** tissue morphology. This can lead to artifacts and poor-quality primary or specialty staining.

# WHAT IS ROTATIONAL AGITATION PROCESSING

Rotational agitation is performed at room temperature and without the use of heat. It offers the advantages of traditional processing by providing an astound improvement in fluid exchange efficiency.

Instead of impeller that moves the processing reagents inside a processing chamber, rotational tissue processor uses a canted (tilted) chamber that rotates baskets with tissue samples in and out of the solutions to induce interaction between the fluids and the specimens.



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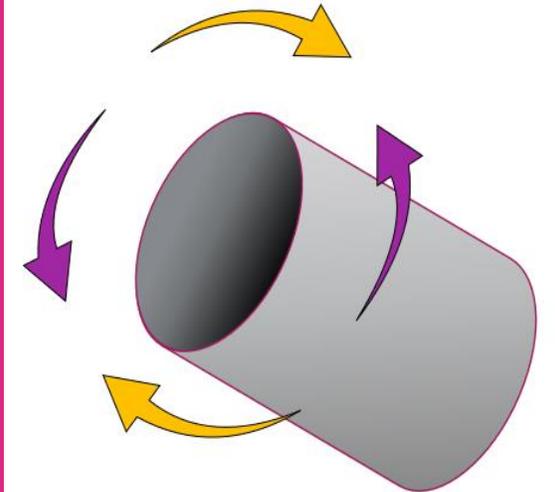
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Rotational Tissue Processor features:

- Smart basket design that allows easy fluid movement in and out of the cassettes



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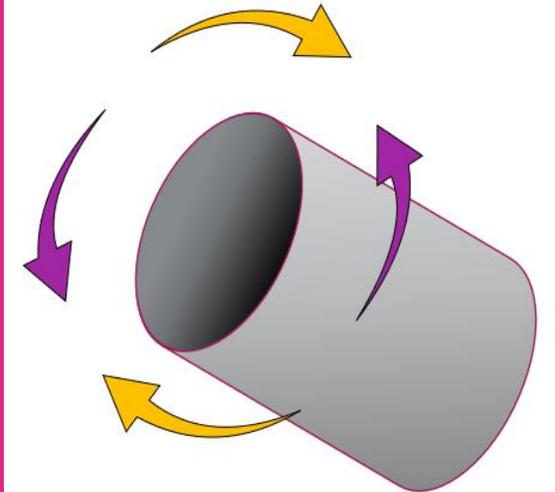
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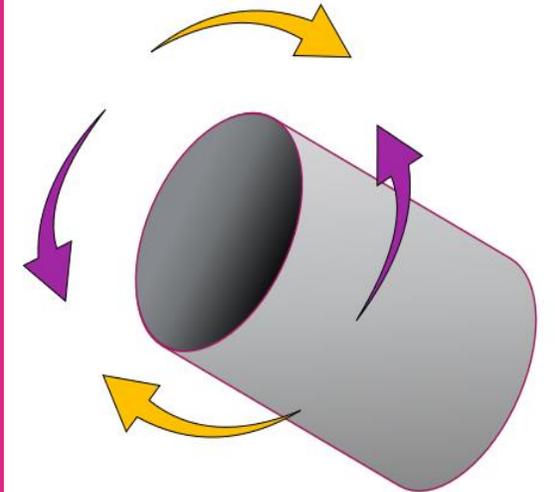
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## Rotational Tissue Processor features:

- Smart basket design that allows easy fluid movement in and out of the cassettes
- Tilted processing chamber
- Partially filling the chamber to allow the tissue to leave the reagent, be briefly suspended out of the reagent and re-submersed into the reagent again.



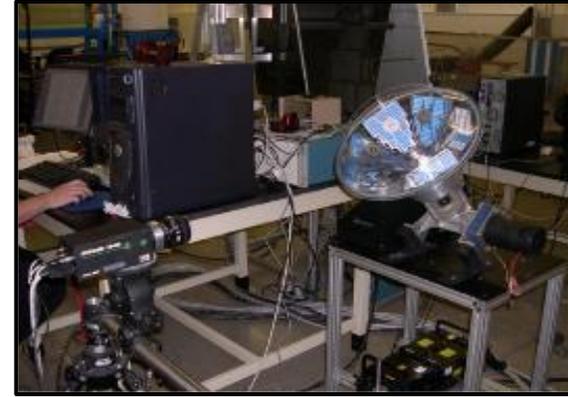
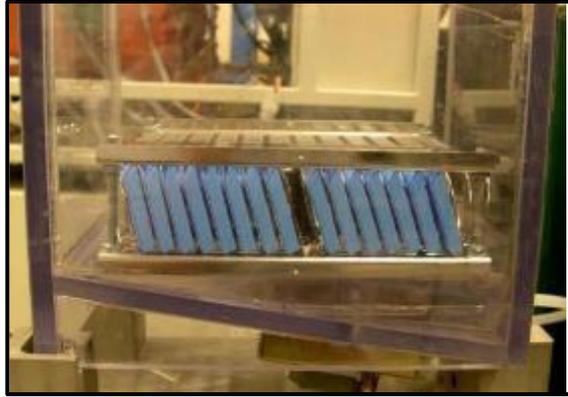
# TIDAL VS ROTATIONAL EXPERIMENTAL DESIGN\*

To compare tidal and rotational agitation methods, models of both types of processors were built for the measurements of:

- Particle Image Velocimetry (flow speed)
- Planar Laser-Induced Fluorescence (flow motion)



Instrumentation & assembly to measure **tidal agitation**



Instrumentation & assembly to measure **rotational agitation**



\* Merati, P et al. *Comparison of tidal agitation and rotary agitation utilizing particle image velocimetry (PIV) and planar laser induced fluorescence (PLIF) methods*. Western Michigan University College of Engineering and Applied Sciences Project Report. 2011

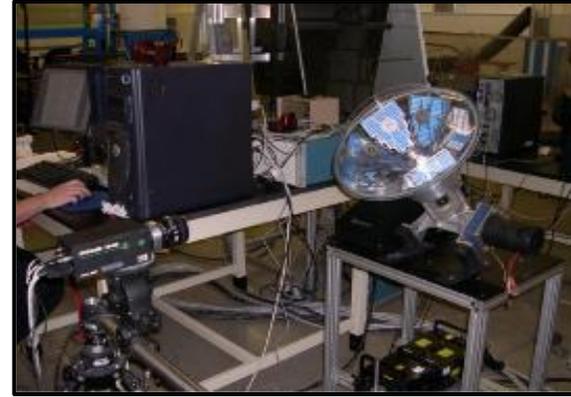
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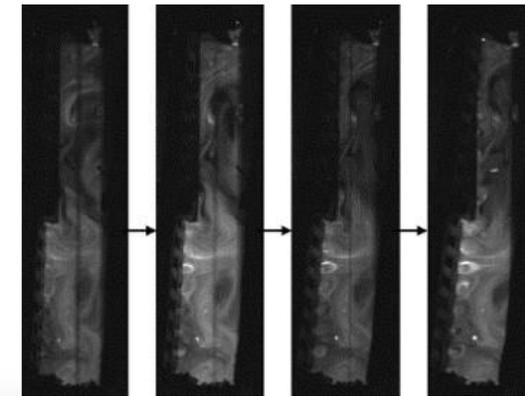


Instrumentation & assembly to measure **tidal agitation**



Instrumentation & assembly to measure **rotational agitation**

Flow visualization studies: *Thanks to rotation, higher magnitude of mixing occurs due to the presence of large turbulence generating vortices as the cassettes are filling up with liquid.*



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# Measuring Efficacy of Rotational Tissue Processor (RTP)

Reagents Flow Speed and Motion measurements within the tissue cassettes were taken for tidal and rotational processors to **compare the flow fields** within the cassettes of each instrument.

These measurements indicated:

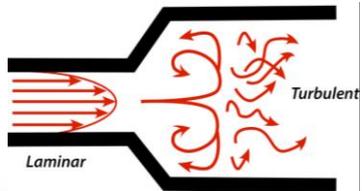
- ✓ Higher flow rates through the cassettes mean that more fluid is passing over the tissue within the cassette during the same time interval, which correlates to faster tissue processing. When compared to traditional tidal agitation processing, RTP provides an overall seven-fold improvement in fluid exchange efficiency.

# MEASURING EFFICACY OF ROTATIONAL TISSUE PROCESSOR (RTP)

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- ✓ Higher flow rates through the cassettes mean that more fluid is passing over the tissue within the cassette during the same time interval, which correlates to faster tissue processing. When compared to traditional tidal agitation processing, RTP provides an overall seven-fold improvement in fluid exchange efficiency.
- ✓ Turbulence and vorticity\* in RTP processor are 5 times larger than the tidal agitation processor. The turbulence and vorticity data would indicate that a good deal of mixing is occurring between the racks.



\* In fluid dynamics:

**Turbulence** or turbulent flow is fluid motion characterized by chaotic changes in pressure and flow velocity.

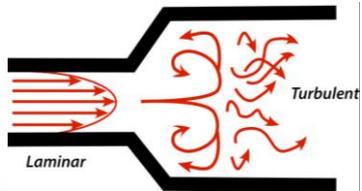
**Vorticity** is the curl of the fluid velocity. It can also be considered as the circulation per unit area at a point in a fluid flow field

# MEASURING EFFICACY OF ROTATIONAL TISSUE PROCESSOR (RTP)

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- ✓ The tidal agitation processor fluid direction is along the cassette length, rather than being perpendicular to it like the RTP processor. This difference can help to reduce the processing time in the RTP model.



\* In fluid dynamics:

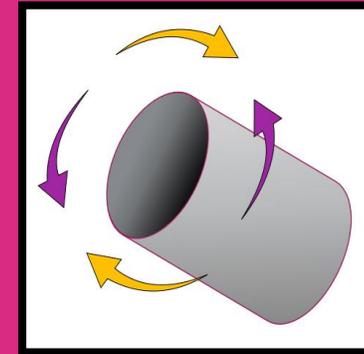
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# ROTATIONAL TISSUE PROCESSOR OFFERS SUPERIOR RESULTS

The rotational tissue processor (RTP) is superior in all ways to the tidal agitation processor:

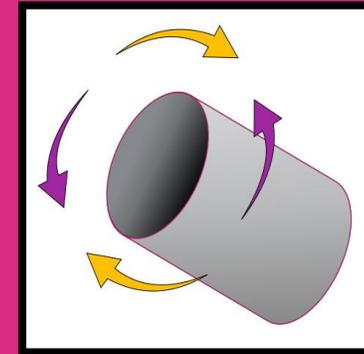
Volume Flow Rate Calculations	
RTP Machine	Tidal Agitation Machine
<b>Drainage Flow Rate</b> Cassette Volume = 3721 mm <sup>3</sup> Drainage Cycles = 8 cycle/min Volume Flow Rate = 496 mm <sup>3</sup> /sec	<b>Drainage Flow Rate</b> Cassette Volume = 3721 mm <sup>3</sup> Drainage Cycles = 0.05 cycle/min Volume Flow Rate = 3 mm <sup>3</sup> /sec
<b>Velocity Flow Rate</b> Cassette Area = 709 mm <sup>2</sup> Average Flow Velocity = 65 mm/s Immersion Time = 40%/cycle Volume Flow Rate = 18434 mm <sup>3</sup> /sec	<b>Velocity Flow Rate</b> Cassette Area = 709 mm <sup>2</sup> Average Flow Velocity = 4 mm/s Immersion Time = 100 %/cycle Volume Flow Rate = 2836 mm <sup>3</sup> /sec
<b>Total Volume Flow Rate = 18930 mm<sup>3</sup>/sec</b>	<b>Total Volume Flow Rate = 2839 mm<sup>3</sup>/sec</b>



# ROTATIONAL TISSUE PROCESSOR OFFERS SUPERIOR RESULTS

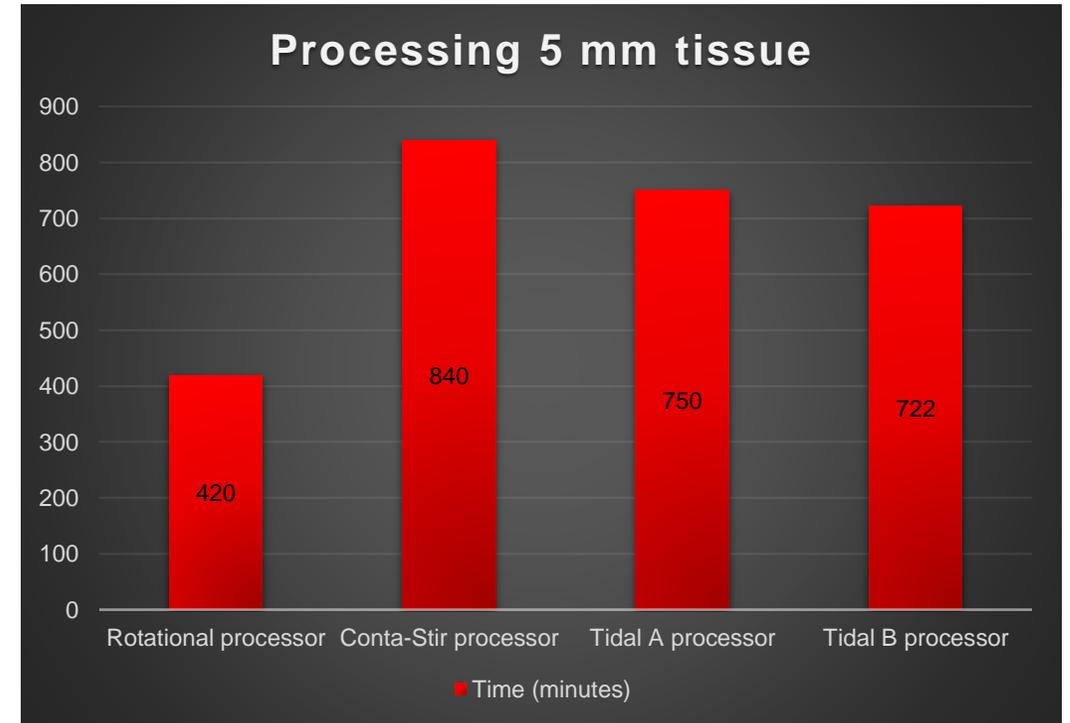
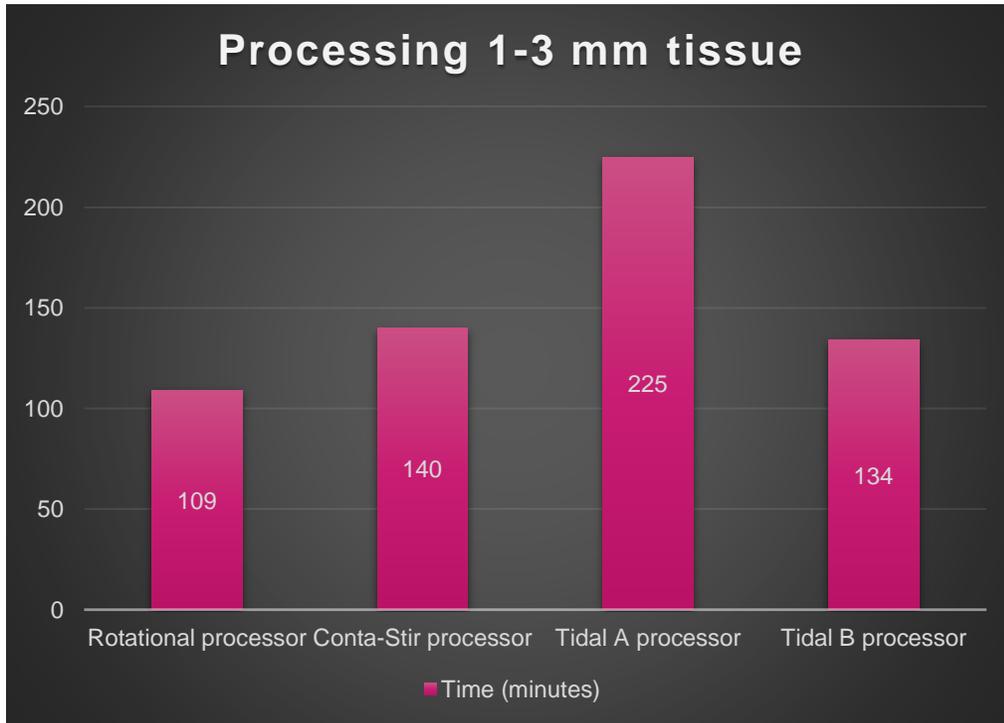
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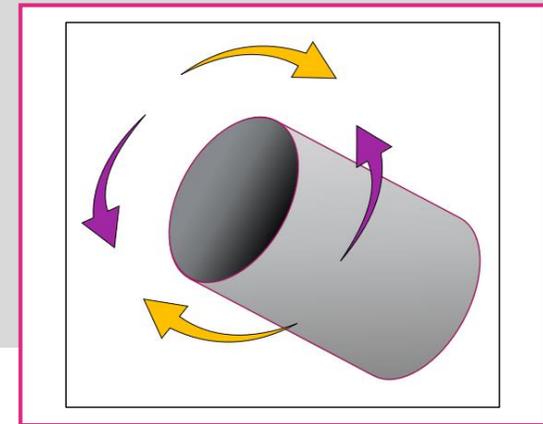
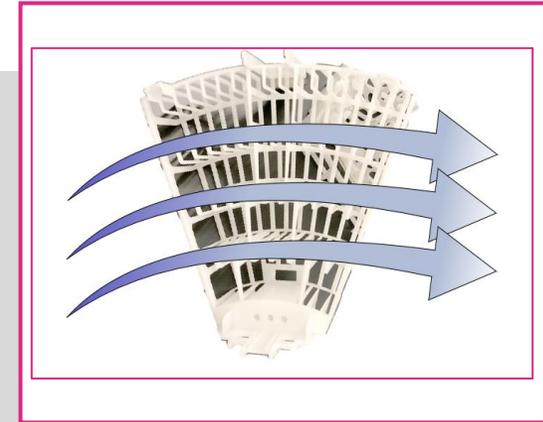
★ - Key differentiators

# PROCESSING PROTOCOL COMPARISON



# WHAT MAKES **AN EFFECTIVE** TISSUE PROCESSOR?

1. The ability to effectively move fluid in-between the cassettes and the baskets
2. The ability to effectively move fluid within the retort chamber
3. Being able to maximize laboratory productivity by rapid processing



# IMPROVE YOUR TISSUE PROCESSING WITH ROTATIONAL PROCESSOR



The EpreDia Revos Tissue Processor, with its unique canted-chamber design, is the **only** premium tissue processor on the market of this kind that allows for rotational agitation within the chamber.

This design enables processing **seven times faster** than the traditional processor without the need for added heat.

